THE INHERITANCE OF HETEROSTYLY IN *LYTHRUM SALICARIA*

E. M. EAST

Harvard University, Bussey Institution, Forest Hills, Massachusetts

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INTRODUCTION

The biology of those plants termed heterostyled by HILDEBRAND was brought into prominence by the publication of DARWIN'S work, "The different forms of flowers on plants of the same species," which appeared first in 1877, and was revised in 1880 and 1884. Darwin was interested in the phenomenon, which is rather widespread, from its bearing on certain problems of evolution; and he carried out a really extraordinary amount of experimental work on the subject, if one makes due allowance for the state of his health at the time.

With his habitual thoroughness, he made numerous observations on a great variety of plants, and established the fact that heterostyly is the extreme manifestation of a graded polymorphism found in various plants usually described as homostyled. These homostyled plants exhibit their weak dimorphism by bearing anthers which differ in color, or by producing pollen differing in color or size. The anthers may also be different in structure. His most extensive experiments, however, were concerned with various dimorphic species of Primula and with trimorphic species of Lythrum and Oxalis.

Though true heterostyly shows variability, like everything else in Nature, the type of polymorphism shown is usually similar in related species. Conditions in *Primula veris*, for example, can be taken as typical of the genus. Here the long styled plants have globular stigmas with long

papillae; they produce oblong pollen measuring .0254 mm at their greatest diameter; they flower earlier than the short styled type, and produce less seed. The short styled flowers have stigmas depressed at the summit, with shorter papillae and bear pollen .038 mm in diameter.

Generally speaking—*Primula auricula* formed an exception—DARWIN found that the mating Long×Short was the most fertile. Short×Long exhibited the next highest fertility. The illegitimate mating Long×Long showed a considerably diminished fertility, while the illegitimate mating Short×Short was least fertile of all. The offspring of Long×Long was practically always Long, though in experiments with *Primula veris* 6 short styled plants (probably due to experimental error) were obtained out of a population of 162. Short×Short yielded both Longs and Shorts; the number of plants obtained, however, was too small to give a dependable ratio.

In *Lythrum salicaria*, DARWIN established a number of interesting facts. The long styled form has a globular stigma larger than those of the other two forms and with longer papillæ. The anthers of the mid-length set are larger than the others. The pollen is the same color in both sets, but that of the mids is larger than that of the shorts. The mid styled form has a long set of stamens with pink filaments bearing purple anthers and green pollen, and a short set with greenish filaments bearing greenish anthers and yellow pollen. The green pollen is larger than the yellow pollen. The short styled form has two sets of stamens much like those of the mid styled form, and bearing like it, green and yellow pollen respectively. The size of the seeds of the three forms is such that five seeds from long styled plants very nearly equal six seeds from mid styled plants or seven seeds from short styled plants.

The legitimate unions, which are cases in which the stigmas are pollinated with pollen from anthers of other flowers borne at the same height, were found to be more fertile than the other or illegitimate unions. Illegitimate unions where long styled or short styled plants were used as mothers were too unproductive to make averages worth while, but mid styled forms fertilized by pollen from the short stamens of the long styled form or by the pollen from the long stamens of the short styled form proved to be almost as productive as legitimate unions.

Long styled plants selfed yielded 56 Longs; short styled plants selfed with mid pollen yielded 8 Shorts and 1 Long; mid styled plants selfed with long pollen yielded 3 Mids and 1 Long. Shorts crossed with Longs (mid pollen) produced 8 Shorts and 4 Longs; Mids crossed with Longs

(short pollen) produced 17 Longs and 8 Mids; Mids crossed with Shorts (long pollen) produced 18 Shorts, 8 Mids, and 14 Longs.

Naturally these results of DARWIN attracted the attention of twentieth century geneticists, and BATESON and GREGORY (1905) were not long in showing that in the Primulas the inheritance of dimorphism may be expressed by the behavior of a single pair of allelomorphs—short style being dominant. Additional experiments with *Primula sinensis* (GREGORY, 1911) showed that there is an association between the factors producing dimorphism and those responsible for the color of the corolla around the tube, which takes the form of a large or a small "eye." A homostyled race with large eye crossed with a short styled race with a small eye yielded F_1 plants having short styles and small eyes. The F_2 generation consisted of 9 short style, small eye: 3 short style, large eye: 3 long style, small eye: 1 homostyle, large eye. Later GREGORY (1915) showed that a short styled plant of *Primula sinensis* behaved as a normal heterozygote when used as a female, but as a homozygote when used as a male. He was not able to determine the reason for this anomalous behavior, however, because the plants used as parents for producing succeeding generations all proved to be homozygous for short style. Still later, GREGORY, WINTON and BATESON (1923) showed that the pair of genes *S* (short style) and *s* (long style) are linked with *B* (blue flowers) and *b* (slaty flowers), *G* (green stigmas) and *g* (colored stigmas), and *L* (stems and leaf-barks light red) and *l* (deep red), in that order.

Simple Mendelian results analogous to those discovered for the Primulas were found to hold for Fagopyrum (CORRENS 1921; DAHLGREN 1922), for Pulmonaria (DAHLGREN 1921), and for Linum (UBISCH 1921; CORRENS 1921; LAIBACH 1922, 1923).

Here the subject of dimorphism rested until ERNST, (1925), from experiments on *Primula hortensis* and *Primula hirsuta*, found that two strongly linked gene pairs are concerned. Short style (*A*) dominates long style (*a*), and long anthers (*B*) dominates short anthers (*b*).

The first post-mendelian work on the inheritance of trimorphism is that of Mrs. BARLOW (1913). The bulk of the data reported in this paper is upon *Oxalis valdiviana*, though a few observations were made upon *Lythrum salicaria*. Mrs. BARLOW found that reciprocal crosses gave like results and therefore that the male and female gametes carry the same factors for flower form, that long styled plants are the ultimate recessives and always breed true to Longs, that mid styled plants give different ratios with the same Short, one yielding no long styled plants and the

other yielding an equal number of Mids and Longs. There was indecisive evidence of a second type of short styled plant yielding a ratio approximating 1 Long:1 Mid:8 Short whereas the usual ratio was 1 Long:1 Mid:2 Short. There was also some evidence of a third type of either Mid or Short. In Oxalis, selfed Shorts produced only Shorts; but in Lythrum all three forms appeared in a family of 10 plants coming from such a union.

Mrs. BARLOW published a second paper, dealing primarily with *Lythrum salicaria*, in 1923. In the meantime, Miss VON UBISCH (1921) offered a two-factor interpretation of trimorphism. Plants *aabb* were assumed to be long styled, plants *aaBB* or *aaBb* mid styled, and all plants containing the factor *A* (with or without *B*) short styled. The publication of this paper is an example of a growing practice, not only among geneticists but among other scientists as well, which is of doubtful value. Mrs. BARLOW had used this factorial interpretation as a working hypothesis before the publication of her first paper. This fact she states in her second paper, and I can bear witness to it, for I discussed the matter with BATESON in either 1912 or 1913. It was, of course, the natural hypothesis to use, it being the simplest imaginable. Since there were numerous conflicting data, however, Mrs. BARLOW felt it unwise to suggest the two-factor hypothesis until these objections had been removed. Miss VON UBISCH published the hypothesis nevertheless, *without presenting any experimental work*; and it is now spoken of in the literature as "Ubisch's hypothesis."

Mrs. BARLOW's data can be discussed to better advantage when our own data are presented.

EXPERIMENTAL WORK

Experiments on the inheritance of trimorphism were started at the BUSSEY INSTITUTION OF HARVARD UNIVERSITY about ten years ago, using certain species of Oxalis as material. Several small populations were raised; but as these species proved to be unsuitable for experimental work, the investigations were soon abandoned. HILDEBRAND obtained all three forms from each of the six legitimate unions while working with Oxalis. We were unable to corroborate his work, our ratios being similar to those found in Lythrum. Generally speaking, Mrs. BARLOW's results were also similar to those obtained in Lythrum. The only ratio reported as particularly exceptional being one of 6L:102M:101S, where no Longs were to be expected. But we obtained such ratios in Lythrum where the so-called "homozygous" Mids were used. One is inclined to assume, therefore that experimental errors must have crept into HILDEBRAND's work.

The experiments with *Lythrum salicaria* have been carried on for seven years, both with American material and with material kindly supplied by Mrs. BARLOW in 1920. My thanks are due to Dr. E. S. ANDERSON, Dr. P. C. MANGELSDORF, Dr. R. A. BRINK, Dr. F. G. BRIEGER, and Mr. S. H. YARNELL, who at various times assisted in the work. In addition, I wish to acknowledge my indebtedness to Dr. A. J. MANGELSDORF, who, besides relieving me of much of the drudgery always connected with experiments of this type, helped plan many of the tests.

Self-fertilizations

No selfed seeds from long styled plants were tested. It seemed reasonable to accept as valid the experience of DARWIN and several of his friends and correspondents and of Mrs. BARLOW that Longs selfed with pollen from either the mid stamens or the short stamens give Longs only.

Many efforts were made to self Shorts, but all attempts were unsuccessful. The only data available, therefore, are those of DARWIN and of Mrs. BARLOW. On account of the small numbers involved, all one may say of these results is that selfed Shorts can apparently give either Shorts and Longs, or all three forms. No reliance can be placed on the ratios obtained.

TABLE 1

Results obtained from selfed Shorts.

INVESTIGATOR	UNION	RESULT		
		L	M	S
DARWIN	own *m*	1	0	8
BARLOW	own *l*+*m*	1	1	8
BARLOW	own *l*+*m*	1	1	2
BARLOW	S *l*	2	0	1

Mids are more easily selfed, as all investigators have found. The results of DARWIN and of Mrs. BARLOW are as follows:

TABLE 2

Results obtained from selfed Mids.

INVESTIGATOR	UNION	RESULT		
		L	M	S
DARWIN	own *l*+*s*	1	3	0
BARLOW	own *l*+*s*	0	3	0
BARLOW	own *l*+*s*	3	9	0
BARLOW	own *s*, *l* emas.	1	2	3
BARLOW	own *l*, *s* emas.	28	90	0
BARLOW	own *l*, *s* not emas.	16	74	1

In 1920, Mrs. BARLOW kindly sent me 12 capsules of Mids selfed with their own long pollen. They were from a union that had previously given her 28 Longs and 90 Mids. The plants produced consisted of 47 Longs and 104 Mids, making a total of 75 Longs and 194 Mids from this union,—a noticeable excess of Longs if the ratio to be expected is 1:3. No particular loss of vigor was occasioned by the selfing. The plants, grouped into four classes for vigor, were 9 very low:8 low:26 medium:27 high. Individual capsules yielded the following ratios of Longs to Mids; 13:24, 2:16, 1:4, 11:8, 0:8, 3:10, 4:9, 1:2, 0:7, 0:7, 3:4, and 9:5.

Since selfed Mids, on the two-factor hypothesis, should yield a certain proportion of homozygous Mids, a series of mid styled plants were crossed with pollen from a single presumably "homozygous" Mid (A 19). The selfing in the majority of cases was done with a combination of "long" and "short" pollen; but where the test was made, no difference in result was obtained when either kind was used alone.

A second Mid (D 18) of the same type was also given a thorough test as a male on Mid plants of various origin (table 4). The results were similar to those obtained with Mid A 19.

TABLE 3

Results from crossing Mids coming from selfed Mids with pollen from a so-called "homozygous" Mid of similar parentage.

CROSS	RESULT		
	L	M	S
B6 ×A19	1	19	0
E6 ×A19	0	5	0
N8 ×A19	0	1	0
A33×A19	0	37	0
A28×A19	0	5	0
A6 ×A19	0	4	0
F15×A19	0	1	0
D8 ×A19	0	12	0
B13×A19	0	3	0
G8 ×A19	0	1	0
N8 ×A19	0	5	0
B14×A19	0	5	0
B18×A19	0	2	0
D14×A19	0	1	0
L8 ×A19	0	3	0
L1 ×A19	0	7	0
A39×A19	2	6	0
Total	3	117	0

Both of these Mids, although they produced ratios totally unlike those produced by ordinary Mids when crossed upon other Mids, do not behave like pure homozygotes. Both produce a certain percentage of "*b*" gametes.

TABLE 4

Results from crossing Mids with pollen from a so-called "homozygous" Mid.

CROSS	ORIGIN, FEMALE	RESULT		
		L	M	S
B3 ×D18	Selfed Mid	0	7	0
B13×D18	" "	0	4	0
C3 ×D18	" "	0	2	0
D12×D18	" "	0	7	0
D16×D18	" "	1	31	0
E11×D18	" "	0	22	0
G15×D18	" "	0	1	0
H35×D18	" "	0	3	0
K10×D18	" "	1	19	0
L5 ×D18	" "	3	7	0
N2 ×D18	" "	0	16	0
N8 ×D18	" "	2	10	0
(A6 ×A19)6 ×D18	Selfed Mid×Mid	0	2	0
(A33×A19)2 ×D18	" " " "	0	20	0
(A33×A19)3 ×D18	" " " "	0	21	0
(A33×A19)19×D18	" " " "	0	10	0
(A33×A19)35×D18	" " " "	0	3	0
(B6 ×A19)7 ×D18	" " " "	1	1	0
(B13×A19)1 ×D18	" " " "	0	20	0
(B13×A19)2 ×D18	" " " "	0	25	0
(B14×A19)4 ×D18	" " " "	0	8	0
(B18×A19)1 ×D18	" " " "	2	15	0
(D14×A19)2 ×D18	" " " "	0	19	0
(L1 ×A19)2 ×D18	" " " "	0	2	0
(L1 ×A19)7 ×D18	" " " "	0	8	0
(N8 ×A19)3 ×D18	" " " "	0	40	0
(LA×MA)8 ×D18	Long×Mid	1	6	0
(ME×LE) 80×D18	Mid×Long	1	14	0
(ME×LE) 87×D18	" " "	1	4	0
(ME×LE) 92×D18	" " "	1	31	0
(N14×D) 18	Selfed Mid	1	27	0

NOTE: The female parent is given first. The figure outside of the parentheses is the number of the plant used from the cross cited.

The ratios obtained in the various families appear to be similar, but owing to the small number of plants appearing in each, the point cannot be determined.

Crosses between Long styled and Mid styled plants

A direct measure of the percentage of "*b*" gametes produced by "homozygous" Mids A 19 and D 18 as males is given by using them on Longs. Long LA crossed with Mid A 19 yielded a ratio of 9 L:85 M:0 S. Long LA crossed with Mid D 18 yielded a ratio of 4L :41 M:0 S. The two results are very similar. Another Long, 1220–5, crossed with Mid D 18, yielded a ratio of 1 L:35 M:1 S. Long LA was also crossed with Mid 1028–3 which should be by origin a "homozygous" descendant of Mid D 18. The ratio was 5 L:30 M:0 S. The total results from these four crosses of Longs by "homozygous" Mids were 19 L:191 M:1 S. The Short, which was one of two produced by a large series of matings between Longs and Mids, can probably be disregarded as the effect of a stray pollen grain. There were 19 Longs out of 210 progeny, thus showing that these "homozygous" Mids produced on the average 9.0 percent "*b*" gametes.

The question arises whether any of the female gametes of these "homozygous" Mids carry the factor "*b*". Four matings are available involving our much used homozygous Mids legitimately crossed with various Longs.

MA 19×LA	produced 1 L:16 M:0 S
MD 18×LA	produced 4 L:64 M:0 S
MD 18×L 1220–2	produced 0 L:14 M:0 S
MD 18×L 1220–5	produced 4 L:27 M:0 S

The two Mids, A 19 and D 18 gave exactly the same results when pollinated with mid pollen from the same Long. From these two crosses, it appears that 6.7 percent of the female gametes of the Mids carry the factor "*b*"; but if all four are averaged, this percentage rises to 6.9.

In addition, a "homozygous" Mid, 1208–21, was crossed with the mid pollen of Long 1202–49, and produced a family with the ratio 1 L:94 M: 0 S. Another apparently "homozygous" Mid, 1208–5 crossed with the same Long, 1202–49, produced a family with the ratio 13 L:88 M:0 S. Perhaps the last two Mids regularly produce 1.0 percent and 13.0 percent of "*b*" gametes respectively. On the other hand, these results may be merely extremes that should be averaged with the other crosses. If this is done, the total is 23 L:303 M, or a little over 7.0 percent of "*b*" gametes.

In four crosses Longs were pollinated with pollen from "heterozygous" Mids. The first three were legitimate unions, the last was illegitimate.

LA×MA	produced 13 L:20 M:0 S
LA×M14	produced 31 L:21 M:0 S
LA×M(LA×M14)	produced 1 L: 2 M:0 S
LA×MA	produced 8 L: 3 M:0 S
	Total 53 L:46 M:0 S

Apparently this ratio approaches 1 L:1 M, and the simplest interpretation is to assume that a recessive has been mated with a heterozygous dominant.

Five other crosses were made with "heterozygous" Mids used as females. All were the result of legitimate unions.

M4×L5	produced	7 L:	11 M:0 S
M(4×5)×L(4×5)	produced	54 L:	39 M:0 S
M(der. 4×5)×L(der. 4×5)	produced	58 L:	55 M:0 S
M(der. above cross with S)×LD	produced	28 L:	21 M:0 S
M 1208–4×L 1202–48	produced	62 L:	57 M:1 S
	Total	209 L:	183 M:1 S

The single Short appearing in these populations may be assumed to be the result of a stray pollen grain. Disregarding it, "heterozygous Mids" crossed with Longs gave populations totaling 209 Longs and 183 Mids. Again there is an excess of Longs, and this excess is what might be expected if the Mids were producing 46.0 percent "*B*" gametes and 53.0 percent "*b*" gametes.

Crosses between Long styled and Short styled plants

In her 1923 paper, Mrs. BARLOW states as one of the objections to the two-factor hypothesis that Longs crossed with Shorts have given only Longs and Shorts, whereas occasional Shorts of constitution *AaBb* should give the ratio 1 L:1 M:2 S when crossed with Longs. Unfortunately, Mrs. BARLOW reported only three crosses between Longs and Shorts. Short 14/4×Long yielded a family 19 L:0 M:18 S. Similarly, Short 14/6×Long produced a family 29 L:0 M:22 S. This result is what might have been expected, for the Shorts involved in these crosses probably do not carry Mid. But in the third cross Short 14/5, which had previously given a 1 L:3 M:4 S ratio (37:121:178) when crossed with a Mid, and therefore should be a Short of constitution *AaBb* on the two-factor hypothesis, also gave a ratio of 1 L:1 S (29 L:0 M:27 S) when crossed with a Long.

We made a large number of crosses between Longs and Shorts partly to determine the ratios produced and partly to establish the constitution of various Shorts that were to be crossed with Mids.

Table 5 reports the results from crossing Longs with Shorts which do not carry Mid, by legitimate unions. The first nine crosses listed were duplicated with illegitimate unions. The total results for these particular crosses are 242 L:0 M:245 S, a very good approximation of a 1:1 ratio

TABLE 5

Results from crossing Longs with Shorts which do not carry Mid.
Legitimate unions

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1106	L×S	leg.	A (wild plant)	C (wild plant)	2	0	4
1111	L×S	leg.	A (wild plant)	(SA×MA)3	39	0	43
1113	L×S	leg.	(M×L)4	C (wild plant)	22	0	22
1115	L×S	leg.	(M×L)4	E (wild plant)	19	0	10
1117	L×S	leg.	(M×L)5	B (wild plant)	30	0	11
1126	L×S	leg.	(M×L)63	A (wild plant)	72	0	67
1128	L×S	leg.	(M×L)67	C (wild plant)	22	0	26
1134	L×S	leg.	(M×L)5	C (wild plant)	13	0	12
1203	L×S	leg.	A (wild plant)	((M×L)32×SC)30	33	0	50
1130	L×S	leg.	(M×hom. M, D18)11	(SA×MA)3	6	0	8
1131	L×S	leg.	(M×hom. M, D18)11	(SA×MA)4	2	0	4
1201	L×S	leg.	A (wild plant)	(SA×MA)4	12	0	17
1213	L×S	leg.	A (wild plant)	(M(M×L)32×SE)3	1[1]	0	3
Total for crosses repeated with illegitimate unions					242	0	245
Grand total					263	0	277

of Longs and Shorts. The grand total for these crosses is 263 L:0 M:277 S. The results from duplicates of these nine crosses made by illegitimate unions are reported in table 6. The results are 123 L:0 M:127 S, thus showing that the products of illegitimate and of legitimate unions are similar. (Results of legitimate and of illegitimate crosses are also similar in vigor. Inbreeding reduced vigor, not illegitimate mating.) Adding Cross No. 1105, an illegitimate union not duplicated by a legitimate union, the distribution of the progeny is 128 L:0 M:136 S.

Four crosses were also made by legitimate unions, in which the Shorts were used as the females and the Longs as the males. They are reported in table 7. Cross No. 1215 is the reciprocal of No. 1105 (illeg.). No reciprocals of the other three crosses were made, but some of the crosses in tables 5 and 6 are with parents of similar derivation. The total results are 46 L:0 M:68 S. If this ratio is assumed to be a 1 L:1 S ratio, there is clearly a deficiency of Longs.

TABLE 6

Results from crossing Longs with Shorts which do not carry Mid.
Illegitimate unions

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1107	L×S	illeg.	A (wild plant)	C (wild plant)	5	0	6
1112	L×S	illeg.	A (wild plant)	(SA×MA)3	3	0	1
1114	L×S	illeg.	(M×L)4	C (wild plant)	42	0	48
1116	L×S	illeg.	(M×L)4	E (wild plant)	25	0	21
1118	L×S	illeg.	(M×L)5	B (wild plant)	22	0	27
1127	L×S	illeg.	(M×L)63	A (wild plant)	1	0	3
1129	L×S	illeg.	(M×L)67	C (wild plant)	11	0	13
1135	L×S	illeg.	(M×L)5	C (wild plant)	8	0	3
1203a	L×S	illeg.	A (wild plant)	(M(M×L)32×SC)30	6	0	5
1105	L×S	illeg.	A (wild plant)	A (wild plant)	5	0	9
Total for crosses repeated with legitimate unions					123	0	127
Grand total					128	0	136

Nearly all of the Shorts listed as plants which do not carry Mid were wild plants from the vicinity of Boston (numbered with a letter). From the high frequency of Longs in the places where they were found and

TABLE 7

Results from crossing Shorts which do not carry Mid with Longs.

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1215	S×L	leg.	A (wild plant)	A (wild plant)	3	0	4
1223	S×L	leg.	C (wild plant)	(M(M×L)5×SC)6	16	0	24
1224	S×L	leg.	C (wild plant)	(LA×SC)7	11	0	22
1225	S×L	leg.	C (wild plant)	(M(M×L)5×SC)12	16	0	18
Total					46	0	68

from the relative ease of legitimate unions and the relative difficulty of illegitimate unions, it was hardly to be expected that they would carry Mid. The Shorts crossed with Longs reported in table 8, on the other hand, were all derived from crosses with Mids. It was to be expected that many Shorts of such derivation would carry Mid. In table 8, 11

TABLE 8

Results from crossing Shorts carrying Mid with Longs.

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1109	L×S	leg	A (wild plant)	(SA×MA)2	3	2	5
1110	L×S	illeg.	A (wild plant)	(SA×MA)2	4	1	5
1205	L×S	leg.	A (wild plant)	(M(M×L)32×SC)37	11	16	15
1207	L×S	leg.	A (wild plant)	(M(M×L)32×SC)2	6	4	6
1207a	L×S	illeg.	A (wild plant)	(M(M×L)32×SC)2	1	0	2
1209	L×S	leg.	A (wild plant)	(M(M×L)32×SE)1	3	0	6
1211	L×S	leg.	A (wild plant)	(SA×MA)2	16	16	41
1211a	L×S	illeg.	A (wild plant)	(SA×MA)2	2	0	1
1226	L×S	leg.	A (wild plant)	(M(M×L)32×SC)1	1	3	6
1303	L×S	leg.	(LA×S(SA×MA)2)70	(SA×hom. M(D18))43	5	10	21
1309	L×S	leg.	(LA×S(SA×MA)2)70	(SA×MA)2	1	2	4
1301	S×L	leg.	(SA×hom. M(D18))43	(LA×S(SA×MA)2)70	25	24	76
1306	S×L	leg.	(SA×hom. M(A19))2	(LA×S(SA×MA)2)70	4	51	70
1311	S×L	leg.	(MA×(M(M×L)32×SC)37)5	(MA×(SA×MA)4)48	17	37	37
1312	S×L	leg.	(MA×(M(M×L)32×SC)37)6	(MA×(SA×MA)4)49	5	26	46
Total L×S					53	54	112
Total S×L					51	138	229

such crosses are given but three of them are merely duplicates of other crosses made by illegitimate unions. The ratio exhibited by these populations is 53 L:54 M:112 S, a rather exact 1:1:2 ratio. In nine of these crosses the female parent was the same, the wild Mid plant A. In the other two crosses, the female parent was a Long coming from a cross between a wild Long plant A and a Short known not to carry Mid because of previous tests. The male parents, the Shorts, all but one came from crosses between Mids supposed to be the "heterozygous" type with Shorts known not to carry Mid. The one exception was a Short derived from a cross between a Short known not to carry Mid and a "homozygous" Mid, D18.

There is no reason to believe that the illegitimate unions give results different from those of the legitimate unions. Only 1 Mid was obtained from the three illegitimate unions, it is true; but this result is not extraordinary since the number involved is small (7L:1M:8S).

It may also be noted that one cross, No. 1209, is listed as carrying Mid although no Mids were produced. Only 9 plants make up this population, and it was thought proper to list the family in this table because the Short

parent used showed that it carried Mid in Cross No. 1210, reported in table 10.

The crosses where the Shorts were used as females, show even greater variability than when they were used as males. The four populations shown total 51 L:138 M:229 S. This distribution of the three forms can hardly be a 1:1:2 ratio by any stretch of the imagination, especially when one considers that the families are large, that seed production was high and that seed germination was a little better than average, (50–85 percent). Clearly there are two distinct ratios here. Population No. 1301 is a straight reciprocal of population No. 1303. By the two-factor hypothesis the Short parent can only be *AaBb*; therefore it should and does give a possible though bad 1:1:2 ratio (25:24:76). Population No. 1306 is practically a duplicate in origin of population No. 1301, however, and it obviously does not exhibit a 1:1:2 ratio, (4:51:70). In the other two populations, No. 1311 and No. 1312, the Short parents may be either *AaBB* or *AaBb* on the two-factor hypothesis. They arose from crossing "heterozygous" Mids with Shorts that might have carried Mid. In other words, under the two-factor hypothesis the Short parents arose by cross *aaBb*×*AaBb*, and therefore may be either *AaBB* or *AaBb*. The ratios do not have to be of the same type under this supposition, but again we are called upon to explain the "*b*" gametes that "homozygous" Mids appear always to produce.

Crosses between Mid styled and Short styled plants

Mrs. BARLOW obtained the largest number of families whose ratios were discordant with those to be expected on the two-factor hypothesis from crosses between Mids and Shorts. She started with a single Short and a single Mid which in reciprocal crosses gave families with the three forms in the ratio 57 L:56 M:139 S. This parental Short crossed again with offspring Mids produced 169 L:195 M:356 S. It is possible to look upon these ratios as the 1:1:2 ratios which should be obtained by crossing Shorts *Aabb* with Mids *aaBb*.

Since some of the Shorts derived from the second group of crosses should be *AaBb*, six chosen at random were crossed back with the original Mid. One produced a family having the distribution 31 L:40 M:87 S,—not a bad 1:1:2 ratio. The other five produced families totaling 87 L: 313 M:382 S,—a possible 1:3:4 ratio. Five shorts carrying Mid to one not carrying Mid where equal numbers are to be expected, appears an unlikely occurrence to Mrs. BARLOW; though it is not without the bounds of possibility.

More disquieting for the two-factor hypothesis, as the author notes, are the following cases. A single Short gave a 1:1:2 ratio (28 L:30 M: 64 S) with one Mid, and a 1:3:4 ratio (20 L:57 M:63 S) with another Mid. Second, a Short proved not to carry Mid by a cross with a Long, produced 84 L:97 M:137 S when crossed with a heterozygous Mid. Mrs. BARLOW believes that this deficiency of Shorts is serious. More contradictory still is the third case. A Short giving a 1:3:4 ratio (37 L: 121 M:178 S) when crossed with a Mid, gave 29 L:0 M:27 S when crossed with a Long. Thus this Short, which should be *AaBb* by the first cross, appears to be *Aabb* by the second.

In our own crosses some similar difficulties with the two-factor hypothesis were found, but they appear to be concerned only with the factor postulated as "*B*." And even where Shorts known not to carry Mid were crossed with Mids, some very fair 1:1:2 ratios were obtained. Nine crosses between Mid females and Short males, and one cross between a Short female and a Mid male, are reported in table 9. Summation of these populations gives the figures 126 L:125 M:243 S.

On the other hand, when it comes to crossing Mids with Shorts which, through crosses with Longs are known to carry Mid, there is practically no evidence for 1:3:4 ratios. The seven crosses between Mid females and Short males, reported in table 10, give populations totaling 22 L:

TABLE 9

Results from crossing heterozygous Mids with Shorts known not to carry Mid.

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1120	M×S	leg.	M(M×L)32	C (wild plant)	13	16	36
1121	M×S	illeg.	M(M×L)32	C (wild plant)	1	2	2
1124	M×S	illeg.	M(M×L)53	E (wild plant)	18	13	14
1136	M×S	leg.	M(M×L)32	E (wild plant)	17	21	36
1137	M×S	leg.	M(M×L)32	E (wild plant)	2	3	6
1202	M×S	leg.	A (wild plant)	(SA×MA)4	28	31	48
1204	M×S	leg.	A (wild plant)	(M(M×L)32×SC)30	3	3	17
1214	M×S	leg.	A (wild plant)	(M(M×L)32×SE)3	17	8	35
1227	M×S	leg.	A (wild plant)	(SA×MA)3	3	7	5
1216	S×M	leg.	A (wild plant)	A (wild plant)	24	21	44
Total					126	125	243

151 M:188 S,—a serious deficiency of Longs. A similar deficiency occurs when Short females are crossed with Mid males. The two crosses made gave a total of 22 L:95 M:139 S. Altogether, in these nine populations

there were 44 L:246 M:327 S. Only one family, as may be seen by reference to the table, gave a fair 1:3:4 ratio. Cross No. 1302 yielded 20 L:54 M:71 S. The remaining families show a great deficiency of Longs and an excess of Mids over what is demanded by the two-factor hypothesis.

TABLE 10

Results from crossing heterozygous Mids with Shorts known to carry Mid.

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1206	M×S	leg.	A (wild plant)	(M(M×L)32×SC)37	0	12	15
1208	M×S	leg.	A (wild plant)	(M(M×L)32×SC)2	3	17	13
1212	M×S	leg.	A (wild plant)	(SA×MA)2	3	24	21
1210*	M×S	leg.	A (wild plant)	(M(M×L)32×SE)1	7	37	40
1308	M×S	leg.	(hom. M(D18)×LA)17	(SA×MA)2	4	7	12
1321	M×S	leg.	(hom. M(D18)×LA)17	(M(M×L)32×SC)37	3	8	21
1304	M×S	leg.	(hom. M(D18)×LA)12	(SA×hom. M(A19)2	2	46	66
1305	S×M	leg.	(SA×hom. M(A19))2	(hom. M(D18)×LA)12	2	41	68
1302	S×M	leg.	(SA×hom. M(D18))43	(hom. M(D18)×LA)17	20	54	71
Total					44	246	327

* Short not *known* to carry Mid.

The only other bit of evidence available is shown in table 11, where the results from crossing Shorts known not to carry Mid with "homozygous" Mids are listed. The two populations have a total of 5 L:36 M:45 S.

TABLE 11

Results from crossing Shorts known not to carry Mid with "homozygous" Mids.

NUMBER	CROSS	UNION	ORIGIN, FEMALE	ORIGIN, MALE	FORM OF PROGENY		
					L	M	S
1217	S×M	leg.	A (wild plant)	hom. M(D18)	4	30	37
1218	S×M	leg.	A (wild plant)	hom. M(A19)	1	6	8
Total					5	36	45

According to the two-factor hypothesis, 1 M:1 S should be the result. Again, the supposedly "homozygous" Mids, D18 and A19, are shown to be producing in the neighborhood of 10 percent "*b*" gametes.

DISCUSSION

My interest in heterostyly was aroused originally by its apparent relationship to self-sterility, a subject upon which I have been working

more years than I like to recall. In *Lythrum salicaria*, short styled plants are almost completely self-sterile. DARWIN and BARLOW have raised 26 plants from selfed Shorts. I have obtained none,—no seeds being formed even on vigorous isolated plants. Selfed Longs produce seeds more frequently; selfed Mids are the most prolific of all. Yet even so, the species may be classed as a self-sterile species. It seemed quite probable, therefore, that the behavior of trimorphic plants would be interpretable as self-sterility linked in some manner to morphological differentiation of the flowers.

Unfortunately the genetic interpretation of self-sterility based upon selective pollen tube growth that has been found adequate for certain species of Nicotiana, Antirrhinum and Veronica does not fit this case. There is differential pollen-tube growth, as will be shown in a later paper; but there is no evidence, either of intra-sterile groups of plants among those having the same flower form or of genetic factors controlling self-sterility linked with flower-form differences, that is at all comparable with the situation in Nicotiana, Antirrhinum and Veronica. Mids and Shorts have two kinds of pollen differing in color, size and in nutrient content (starch or fat) and even Longs have two different sizes of pollen; nevertheless the two kinds of pollen of a particular plant bear the same genetic factors for heterostyly,—a matter proved by the identical results of legitimate and illegitimate unions. None of the ordinary tests useful in proving the genetic behavior of ordinary self-sterile plants, in fact, give us information on the genetics of self-sterility in Lythrum. A physiological interpretation may be found which will fit both types, but a genetic interpretation is unlikely.

The genetic interpretation used as a working hypothesis during the earlier part of these experiments,—the two-factor scheme by which Longs are represented by the formula *aabb*, Mids by the formulæ *aaBb* and *aaBB* and Shorts by factor *A* epistatic to *B*,—comes just near enough to the facts to make comparisons interesting. The evidence for the case is summarized in table 12. One can hardly examine this evidence without feeling that the two-factor hypothesis is an approach to the truth. Yet it is not the whole truth. There are discrepancies that demand another interpretation. The Long is evidently the ultimate recessive. A single epistatic factor apparently accounts for the Short. But wherever the factor or factors responsible for the Mid condition occur, results are irregular. Selfed Mids produce more Longs than are needed for a ratio of 1L:3M. Presumably "homozygous" Mids throw Longs, that is, they always produce the recessive gametes, even in crosses with Shorts.

Again, there is a marked deficiency of Longs, and in some cases an excess of Shorts, in crosses which, from the derivation of the parents, ought to be *AaBb*×*aaBb* or *aaBb*×*AaBb*. There is also the question of whether

TABLE 12

Summary of tests of the two-factor interpretation of trimorphism in Lythrum salicaria.

TEST	RESULT EXPECTED	RESULT OBTAINED
Selfed Longs (*aabb*)	All Longs	Satisfactory
Selfed Mids (*aaBb*)	1L:3M	Excess Longs
Mids (*aaBB*)×Mids (*aaBb*)	All Mids	Unsatisfactory. Longs produced
Selfed Shorts (*Aabb*)	Longs and Shorts	Satisfactory. No good ratio
Selfed Shorts (*AaBb*)	All three forms	Satisfactory. No good ratio
Longs (*aabb*)×Mids (*aaBb*)	1L:1M	Satisfactory. Excess Longs
Mids (*aaBb*)×Longs (*aabb*)	1L:1M	Satisfactory. Excess Longs
Longs (*aabb*)×Mids (*aaBB*)	All Mids	Unsatisfactory. Longs produced
Mids (*aaBB*)×Longs (*aabb*)	All Mids	Unsatisfactory. Longs produced
Longs (*aabb*)×Shorts (*Aabb*)	1L:1S	Satisfactory
Shorts (*Aabb*)×Longs (*aabb*)	1L:1S	Unsatisfactory. Excess Shorts
Longs (*aabb*)×Shorts (*AaBb*)	1L:1M:2S	Satisfactory
Shorts (*AaBb*)×Longs (*aabb*)	1L:1M:2S	Unsatisfactory. Deficiency Longs
Mids (*aaBb*)×Shorts (*Aabb*)	1L:1M:2S	Satisfactory
Shorts (*Aabb*)×Mids (*aaBb*)	1L:1M:2S	Satisfactory
Mids (*aaBb*)×Shorts (*AaBb*)	1L:3M:4S	Unsatisfactory
Shorts (*AaBb*)×Mids (*aaBb*)	1L:3M:4S	Unsatisfactory. Variable ratios
Shorts (*Aabb*)×Mids (*aaBB*)	1M:1S	Unsatisfactory. Longs produced

or not the elimination of gametes and zygotes commonly found has a genetic significance. The six kinds of pollen found in the three forms exhibit from 10 to 30 percent of abortive grains. This fact may not have a bearing on the genetic interpretation of heterostyly since the distribution of percentages of abortive pollen within each type shows high variability. The occurrence of non-viable zygotes seems more important. Counts of seeds in hundreds of capsules, taken together with germination tests, *show that the same plants used as females regularly eliminate more zygotes in some crosses than in others.*

Not having the seed count and the germination data segregated according to type of cross, I very stupidly overlooked the obvious import of these facts. The common Mendelian schemes were tried,—additional factors, linkage, multiple allelomorphs; but such hypotheses raised up more obstacles than they cleared away. Cytological examinations of all three forms were made, and TISCHLER'S work (1917, 1918), showing 24 pairs of chromosomes for each type with no abnormal behavior, was confirmed. Thus there was no good ground for assuming non-disjunction

or unequal crossing over, even if such assumptions could have been fitted to the facts. But the obvious interpretation presented by the germination tests, balanced lethals, was overlooked until Dr. P. W. WHITING suggested that I try such a scheme.

Retaining the idea that the Long is the ultimate recessive and that the Short is conditioned by an epistatic factor "*A*," let us assume that the Mid is conditioned by duplicate linked factors M_a and M_b that are lethal in the homozygous condition. Owing to the various possibilities of mating, four kinds of Mids can exist, namely (1) $\frac{M_aM_b}{m_am_b}$, (2) $\frac{M_am_b}{m_aM_b}$, (3) $\frac{M_am_b}{m_am_b}$ and (4) $\frac{m_aM_b}{m_am_b}$. The first two types probably ought to be more frequent in Nature than the last two.

For several reasons, among which is the probability that Mids of the constitution $\frac{M_aM_b}{m_am_b}$ are more easily selfed, we may assume that the population produced by our selfed Mids came from a mating of this type. Solving the proper algebraical equation for a crossover percentage that will fit the ratio of 75 L:194 M obtained by this union, we get 10 percent. A 10 percent crossover value will give us 28.8 L:71.2 M. Mids of constitution $\frac{M_aM_b}{m_am_b}$ will form 57.7 percent of the progeny, those of constitution $\frac{M_am_b}{m_am_b}$ and $\frac{m_aM_b}{m_am_b}$ will form 12.8 percent of the progeny, while those of constitution $\frac{M_am_b}{m_aM_b}$ will form 0.71 percent of the progeny. Mids of constitution $\frac{M_am_b}{m_am_b}$ or $\frac{m_aM_b}{m_am_b}$ selfed will give 1 L:2 M, like the yellow mice. Mids of the constitution $\frac{M_am_b}{m_aM_b}$ selfed will give L 0.5:M 99.5, the Mids produced being 80.5 percent of type 2, 9 percent each of types 3 and 4 and 1 percent of type 1. This type of Mid, $\frac{M_am_b}{m_aM_b}$, is our supposedly "homozygous" Mid such as is found in plants D18 and A19.

We may take it then that the crosses shown in table 3 and 4 are crosses in which the females are partly $\frac{M_aM_b}{m_am_b}$ (Cross 1), partly $\frac{M_am_b}{m_aM_b}$ (Cross 2), while the males are $\frac{M_am_b}{m_aM_b}$. Cross 1 yields 4.3 percent Longs; Cross 2,

like the selfing of Mid of Type 2, yields 0.5 percent Longs. It is possible, though not highly probable from their derivation, that some of these crosses also involved females of constitution $\frac{M_a m_b}{m_a m_b}$ and $\frac{m_a M_b}{m_a m_b}$. If so, the results would not be changed substantially, as these types mated with $\frac{M_a m_b}{m_a M_b}$ yield 3.3 per cent Longs. In the first series the result was 3L:117M: In the second series the total was 15 L:507 M. The result was just a little under 3.0 percent Longs, therefore, in each case.

Considering now the crosses between Mids and Longs, we have four possibilities. The common type of "heterozygous" Mid is naturally $\frac{M_a M_b}{m_a m_b}$. This type, when crossed with Long (continuing to use the 10 percent crossover value), yields 45 percent L:55 percent M. In our experiments we obtained 53.3 percent L:46.7 percent M. This result is hardly a good fit, but not sufficiently anomalous to negate the hypothesis, I think. The other two types of "heterozygous" Mid, the rarer types, should give 1 L:1 M when crossed with Longs. If any such plants should have been present in the tests, it would tend to lessen the slight departure from the expected ratio.

The so-called "homozygous" Mids, $\frac{M_a m_b}{m_a M_b}$, crossed with Longs give 5 percent L:95 percent M. We obtained 8 percent Longs in a total of 636 plants. Leaving out one extreme family which produced 13 L to 88 M, the ratio was 7 percent Longs.

The present assumptions make no change in the expected ratio for crosses between Longs and Shorts which do not carry Mid. The ratio should be 1 L:1 S, as was found in the crosses reported in tables 5, 6 and 7. There is a slight excess of Shorts (46 L:68 S) in table 7, but no more than would be expected rather frequently in families of 114.

So also the presumably rarer types of Short, $\frac{A}{a}\,\frac{M_a m_b}{m_a m_b}$ and $\frac{A}{a}\,\frac{m_a M_b}{m_a m_b}$, will give 1 L:1 M:2 S ratios when crossed with Longs, just as in the simple two-factor hypothesis. The common "heterozygous" Short, $\frac{A}{a}\,\frac{M_a M_b}{m_a m_b}$, crossed with Long, should give a ratio of 22.5L :27.5M: 50S. The so-called "homozygous" Short, $\frac{A}{a}\,\frac{M_a m_b}{m_a M_b}$, crossed with Long should give a ratio of 2.5 L:47.5 M:50 S.

Now if we examine table 8, where such crosses are listed, we see that there are two distinct ratios. Totaling the figures of all the crosses except No. 1306 and 1312, gives 95 L:115 M:225 S. This is a percentage ratio of 21.8 L:26.4 M:51.8 S, which is a very satisfactory fit for the first ratio. The other two crosses yielded 9 L:77 M:116 S, a percentage ratio of 4.4 L:38.1 M:57.5 S, only a slight deficiency of Mids from expectation for the second ratio.

Crosses 1301 and 1311, presumably from Shorts carrying "heterozygous" Mid and Crosses 1306 and 1312, presumably from Shorts carrying "homozygous" Mid, are to be regarded as critical tests. From their derivation, they should, on the two-factor hypothesis, give similar results; on the balanced-lethal interpretation they may give different results because of crossovers. On the two-factor hypothesis, for example, "homozygous" Mids A19 and D18 should be *aaBB* and crossed with the same Short should give only Shorts *AaBb*; on the "balanced-lethal" interpretation the same cross may give Shorts $\frac{A}{a}\,\frac{M_a M_b}{m_a m_b}$ and $\frac{A}{a}\,\frac{M_a m_b}{m_a M_b}$.

A great many kinds of matings are possible between Mids and Shorts. We may neglect those where either the Mids or the Shorts contain the formula $\frac{M_a m_b}{m_a m_b}$ or $\frac{m_a M_b}{m_a m_b}$. In the first place they would give results approaching those of the two-factor hypothesis and of certain other cases calculated on the balanced-lethal hypothesis; in the second place, plants of these constitutions should be rare from theoretical considerations and are actually rare as shown by the analysis of our results. For example, a Mid of this type crossed with a Short not carrying Mid gives a 25 L: 25 M:50 S ratio, crossed with a "heterozygous" Short gives a 15 L: 35 M:50 S ratio, crossed with a "homozygous" Short gives a 1.7 L: 48.3 M:50 S ratio, and crossed with a short like itself except for the additional factor A gives a 16.7 L:33.3 M:50 S ratio.

We are interested primarily in the ratios expected when "heterozygous" and "homozygous" Mids are crossed with the three common types of Short.

"Heterozygous" Mids, $\frac{a}{a}\,\frac{M_a M_b}{m_a m_b}$, crossed with Shorts $\frac{A}{a}\,\frac{m_a m_b}{m_a m_b}$, the Shorts which do not carry Mid, give a 22.5 L:27.5 M:50 S ratio. The same Mids crossed with "heterozygous" Shorts, $\frac{A}{a}\,\frac{M_a M_b}{m_a m_b}$, give a 14.4 L: 35.6 M:50 S ratio. When crossed with "homozygous" Shorts, $\frac{A}{a}\,\frac{M_a m_b}{m_a M_b}$, these Mids give a ratio of 2.2 L:47.8 M:50 S.

"Homozygous" Mids, $\frac{a}{a}\ \frac{M_a m_b}{m_a M_b}$, crossed with Shorts which do not carry Mid give a ratio of 2.5 L:47.5 M:50 S. When crossed with "heterozygous" Shorts, these Mids give a ratio of 2.2 L:47.8 M:50 S. And finally, when these Mids are crossed with "homozygous" Shorts, they give a ratio of 0.25 L:49.75 M:50 S.

Any Mids other than the so-called "homozygous" Mids when crossed with Shorts known not to carry Mid, yield very nearly a 1 L:1 M:2 S ratio; and this is what was obtained as seen in table 9.

The two crosses shown in table 11 are between Shorts known not to carry Mid and "homozygous" Mids. Theoretically the ratio is 2.5 L: 47.5 M:50 S; actually it is 5.8 L:41.9 M:52.3 S.

The crosses shown in table 10 are not so easy to analyze. We know, however, that the Mid used in the first four crosses is a "heterozygous" Mid, and that the Shorts must be either "heterozygous" or "homozygous." In the first case the ratio should be 14.4 L:35.6 M:50 S; in the second case the ratio should be 2.2 L:47.8 M:50 S. Actually the plants obtained were 13 L:90 M:89 S. The fit is not bad if one assumes that both types of Short were present.

In the next five crosses both the Mids and the Shorts may be either "heterozygous" or "homozygous," *even if two or more are derived from a single source cross*. Little can be said for the ratio exhibited by the two families No. 1308 and No. 1321, giving together the figures 7 L:15 M:33 S. The number of plants is too small. The last three crosses shown in the table form the critical test. Crosses No. 1304 and No. 1305 are reciprocals. Together they gave 4 L:87 M:134 S, a ratio of 1.8 L:38.7 M:59.5 S, which is a reasonable expectancy for a cross in which *either* the Mid or the Short parent is "heterozygous" and the other parent "homozygous." In Cross No. 1302, both parents were derived from the same matings as in the reciprocal cross just described, and in such a way that by the simple two-factor hypothesis the crosses ought to be duplicates of each other. Yet Cross No. 1302 gives quite a different result. The figures are 20 L:54 M:71 S, a ratio of 13.8 L:37.2 M:49.0 S. This is a very good fit for a cross between a "heterozygous" Mid and a "heterozygous" Short. And it is a result to be expected on the balanced-lethal hypothesis from parents of the same derivation that gave the results obtained in Crosses No. 1304 and No. 1305. Under the two-factor hypothesis the discordance is outstanding and inexplicable.

In all the tests therefore, the balanced-lethal hypothesis fits the facts if one allows for the fluctuations due to random sampling and for the obvious roughness of the crossover determination.

SUMMARY

In this paper it is shown that trimorphism in *Lythrum salicaria* is due to three factors. The long styled plant is the ultimate recessive. The mid styled plant is due to duplicate factors in the same linkage group having a crossover value of about 10 percent. These factors are lethal when in the homozygous condition. The mids most commonly found have the constitution $\frac{M_a M_b}{m_a m_b}$. Mids $\frac{M_a m_b}{m_a m_b}$ were also tested in numerous combinations. The short styled plants are conditioned by an independent factor A. They may or may not carry the factors that condition Mid.

There is some evidence that crossing over is slightly less in the female than it is in the male.

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A GENETIC STUDY OF CERTAIN CHLOROPHYLL DEFICIENCIES IN MAIZE[1]

W. A. CARVER

Florida Agricultural Experiment Station, Gainesville, Florida

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INTRODUCTION

The plant pigment, chlorophyll, is without doubt the most important of all known organic substances, for upon chlorophyll depends the life of the plant containing it and consequently the existence of other forms of life which do not have the power of photosynthesis. It appears, therefore, that a fuller knowledge of chlorophyll inheritance, aside from its immediate biological interest, might lead to useful applications in the way of plant improvement.

The present paper is concerned with the genetic relations between several chlorophyll defects and certain other plant and kernel characters in maize, with the view of adding to our knowledge of the germinal constitution in that species.

MATERIAL AND METHODS

These studies have been carried on with material which was kindly furnished by Dr. E. W. LINDSTROM, Dr. G. N. STROMAN, formerly of the

[1] Contribution from the Department of Genetics, AGRICULTURAL EXPERIMENT STATION, IOWA STATE COLLEGE, No. 34.

TEXAS EXPERIMENT STATION, and Mr. M. T. JENKINS of the IOWA EXPERIMENT STATION. The letters L, S, and J, respectively, will be used before the pedigree numbers of such material. The investigations were begun at the UNIVERSITY OF WISCONSIN and completed at IOWA STATE COLLEGE. The writer wishes to acknowledge at this time his indebtedness to Dr. E. W. LINDSTROM, under whose direction these studies have been made; to Dr. L. J. COLE of the UNIVERSITY OF WISCONSIN for his counsel and encouragement during the first year of this work; and to Dr. G. N. STROMAN and Mr. M. T. JENKINS for helpful suggestions and information concerning the origin of certain characters reported herein.

The degrees of linkage in simple dihybrid segregations were calculated by the method described by EMERSON (1916). For the trihybrid ratios in which there were two complementary genes, one being linked to a third gene, the formulae given by BRUNSON (1924) were used for finding the gametic ratio. The formulae are as follows:

$$r = \sqrt{\frac{(AB+3ab)-(Ab+aB)}{18}}$$

$$s = \tfrac{1}{4}\sqrt{AB+Ab+aB+ab-r}$$

The F_2 theoretical ratio was calculated by substituting the found values of r and s in the formulae adopted from EMERSON as follows:

AB	$9r^2+6(2rs+s^2)$
Ab	$3r^2+6(2rs+s^2)$
aB	$3(2rs+s^2)$
ab	$4r^2+(2rs+s^2)$

For ratios expressing linkage between the factors concerned, the closeness of fit (P) was calculated by ELDERTON'S (1901) method supplemented by PEARSON'S (1924) tables. The values of P may range from 0 to 1, measuring the probability of the deviations from the theoretical frequencies being due to errors of random sampling (HARRIS, 1912).

In dihybrid and trihybrid ratios showing independent inheritance of the factors concerned, the parental and non-parental classes were often grouped against one another and the probable error calculated from these two classes. The general formula used was:

$$P.E. = 0.6745\sqrt{p\,q\,n}$$

in which p and q are the two elements of the ratios as, 10:6 or 9:7, and n is the total number of individuals in the experiment.

The crosses

For the convenience of the reader, the crosses whose progenies form the basis of these studies are given here and also in the body of the paper.

The factor *v* occurring in the genotype of most of the crosses represents a seedling chlorophyll deficiency known as virescent in which the chlorophyll development is delayed for a time.

The virescents which are not known to be different from those previously described (DEMEREC 1924) are written without an Arabic numeral. In the following discussion, these characters shall be distinguished only by reference to their origin. It will be seen in cross No. 5 that two virescent factors have been assigned numerals. Evidence is given in the discussion for believing that these factors are different from those reported by DEMEREC.

The crosses may be represented as follows:

Cross No. 1, L 4992–23×S 899–7

$AA\ Cc\ Rr\ S_u\ s_u\ Yy\ W_l\ W_l\ VV\ S_c\ S_c \times$

$AA\ Cc\ rr\ S_u\ S_u\ Y\ Y\ w_l\ w_l\ Vv\ S_c\ s_c.$

Cross No. 2, L 4992–26×S 899–10

$AA\ Cc\ Rr\ S_u\ s_u\ W_l\ W_l\ VV \times$

$AA\ CC\ rr\ S_u\ S_u\ w_l\ w_l\ Vv.$

Cross No. 3, L 4254–38×L 4260–7

$S_u\ S_u\ L_g\ l_g\ R_o\ R_o \times$

$s_u\ s_u\ L_g\ L_g\ R_o\ r_o$

Cross No. 4, L 4263–3×4260–4

$S_u\ S_u\ Vv\ A_n\ a_n\ R_o\ R_o \times$

$s_u\ s_u\ VV\ A_n\ A_n\ R_o\ r_o$

Cross No. 5, 232–11×230–1

$YY\ V_7\ V_7\ VV\ V_6\ v_6 \times$

$y\ y\ v_7\ v_7\ Vv\ V_6V_6$

The different characters symbolized in the above crosses are given in the discussion of types or in that part of the paper which describes the F_2 results.

Those characters not heretofore described are rolled (r_o) seedlings and several new types of virescent (v) seedlings.

DESCRIPTION OF CHARACTERS

Yellowish virescent. One virescent which appeared in the F_2 generation of crosses No. 1 and No. 2 has a distinct yellow color when it first emerges from the soil. Chlorophyll development in this virescent type is similar to that described by LINDSTROM (1918). The young leaves gradually

become green, beginning at the leaf tips. The yellow color can be observed in the leaves and stems until it is hidden by chlorophyll. When the plant is about three weeks old, the first leaf is usually solid green. Newly unfolded seedling leaves gradually become green from their tips downward. When grown under field conditions during the summer of 1924, about one-third of the plants matured. Most of these produced pollen, although no ears were formed. Mature virescent plants were about one-half as tall as their normal sister plants but were nearly full green.

Light virescent ($v_6\ v_6$)

Plants of this type emerge from the soil practically devoid of chlorophyll. Their leaves are about one-half as broad and their stems are noticeably shorter than those of normal sister plants. Some plants develop only a slight amount of chlorophyll on their leaf tips; others may develop one or two streaks of chlorophyll about one-eighth of an inch in width running the entire length of the leaf. Still other plants have considerable chlorophyll, but have chlorophyll deficient streaks on the leaf borders. Pure virescent plants are not able to live beyond the three-leaf stage.

Two medium green virescents ($v_7\ v_7$ *and an associated virescent*)

These two types trace back to ear J 121–4 of U. S. Selection 133. The two virescents are very similar in appearance, being typical virescent types. The type v_7 seems to have slightly more chlorophyll than its associate. Both virescents have a pale green color when emerging from the soil. Under favorable conditions for growth they become green very rapidly, making their classification difficult. Pure virescent plants are able to mature a small ear and produce an abundance of pollen.

Rolled

In the second generation of crosses No. 3 and No. 4 which combined several plant characters, a semi-lethal seedling type occurred. The young plant of this type seems perfectly normal in every respect until the third leaf appears. This leaf has a pale chlorophyll-deficient appearance and remains for a time rather closely rolled. Simultaneously with the appearance of the third leaf, the first and second leaves become pale, slightly corrugated cross-wise, thickened, and rolled upward at the borders. At this stage, in extreme types, all three leaves die, become blackened and fall from the plant, leaving the stem apparently unaffected. Some plants may recover and be indistinguishable from the normal if growing conditions are favorable. In fact, during the summer of 1924 the character did not appear in the expected proportion of three normal plants to one rolled.

Some evidently recovered so quickly that they escaped notice, and a large proportion of them survived throughout the season. Genetically rolled plants were obviously weak, for none of these plants were chosen for self-pollinating.

The rolled plant deviates from the normal in still other features. The primary root of the rolled seedling is larger in diameter and more turgid than that of normal sister plants. There is also an abnormality of the kernel associated with extreme types of the rolled character. The scutellum and adjacent part of the radicle become dark and woody in structure, and the kernel appears as though it were diseased. Seeds from a segregating ear were sterilized with chlorinated lime and grown with sterilized soil and water. With this treatment 51 normal to 19 rolled plants were produced in an F_2 family. HOLBERT, et al (1924), describe a diseased condition of the maize kernel called scutellum rot, which appears to be similar to the kernel condition associated with rolled seedlings. Seed from an ear segregating for rolled was sent to Mr. HOLBERT to be examined. He found that rolled was phenotypically different from scutellum rot.

White-base-leaf.

As previously stated by STROMAN (1924 b), the white-base-leaf character $w_l w_l$ is very variable in the amount of chlorophyll developed and the pattern in which it occurs over the plant.[1] The nature of chlorophyll dispersion in the chlorophyll deficient areas is one quality which is constant in the material studied by the writer. In the areas where the chlorophyll is sparse, it occurs in broken streaks between the leaf veins, giving a streaked or finely dappled appearance to those regions. The same characteristic dispersion occurs on the stems in a less pronounced fashion. Based on the location of chlorophyll deficient areas, the white-base-leaf character may be roughly divided into two types. In one type, the deficiency is primarily at the base of the leaves. In the other type, seedling plants have nearly pure white areas about one-half inch in length covering their leaf tips. Some of these plants assume a zebra-like appearance when half mature. The latter are probably modifications of the zebra seedling character which was described by STROMAN.

Scarred endosperm

The scarred endosperm type (figure 1) which occurred in the second generation from cross No. 1 is similar in general to the scarred endosperm character described by EYSTER (1922). The greatest difference lies in

[1] The present writer referred to this character as brindled in Iowa Acad. Sci. Proc., 31 (1924), p. 129.

the amount of endosperm developed and the deepness of scarring. In the present material, for example, the crown endosperm is very scant in some kernels; consequently, a more extreme wedge shape is assumed by the upper portion, due to the pressure from adjoining kernels. The bases of such kernels are as large as those of the normal. In length, they are about one-third less. The scars themselves are not so deep as those described by EYSTER. But they are more numerous, occurring on all sides

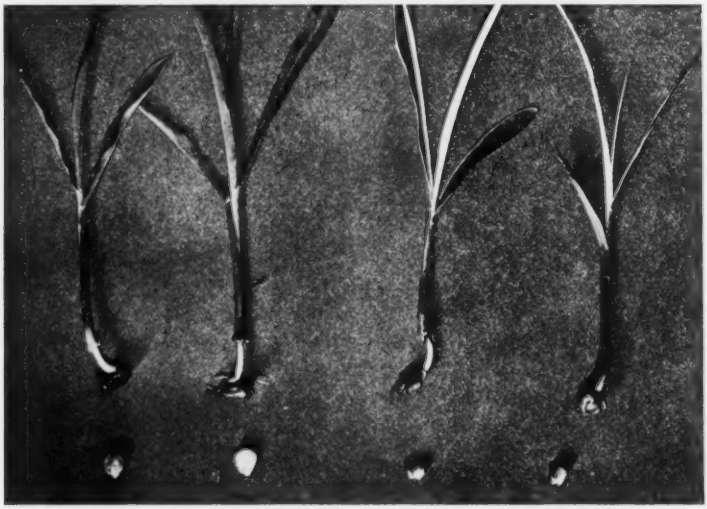

FIGURE 1.—Showing the relation between scarred endosperm and sectored plants. Two normal kernels and plants are shown on the left.

in a minority of the kernels and appear as fine, slightly indented and branched stripes often extending to the kernel base.

INHERITANCE OF CHARACTERS

White-base-leaf

The white-base-leaf character was shown by STROMAN (1924 b) to behave as a recessive character in inheritance. In addition to his counts, the following results have been obtained by self-pollinating F_1 and F_2 green plants heterozygous for white-base-leaf: green seedlings, 2860(2902): white-base-leaf seedlings, 1009(967). The calculated numbers are placed in parentheses. These data support the hypothesis that white-base-leaf occurs as a recessive in a simple 3:1 ratio, (Dev./P.E. = 2.31). This simple relationship is also borne out by two backcrosses which gave 189 green to 161 white-base-leaf plants. The deviation was 14 ± 6.31.

Scarred endosperm and sectorial plants

Scarred endosperm (figure 1) was found by EYSTER (1922) to be a simple Mendelian recessive character. The classification of normal and scarred endosperm in the present material was rather difficult to make; consequently the exact mode of inheritance of this particular scarred endosperm character cannot be ascertained at the present time. The number of F_1 ears showing the scarred endosperm segregation (15) to those which were homozygous normal (11) agrees fairly well with the assumption that the character is determined by a single genetic factor. However, the closest possible classification of the F_2 kernels shows a great deficiency of scarred kernels from the expected number, assuming a monohybrid relationship. The total counts are given in table 15. It is seen that the observed ratio agrees better with a duplicate factor hypothesis, although the deviation then is 3.30 times the probable error.

A very interesting relationship exists between scarred endosperm and a japonica-like seedling type which has been called sectored. The sectored character is evidently linked to scarred endosperm. However, difficult classification of endosperm makes this point uncertain. More will be said concerning this relationship in a later section.

Rolled

The rolled character has proven to be a simple Mendelian recessive to the normal condition. The ratio of non-rolled to rolled plants may be secured from the F_2 tables 3, 4, 5 and 6. The ratio found by summing the counts in these tables is as follows: normal seedlings, 3306 (3272); rolled seedlings, 1057 (1091). The deviation from a 3:1 ratio is 34 ± 19.3.

An F_3 progeny test of 48 ears from normal F_2 plants furnishes further evidence as to the simple monohybrid relationship of the rolled character. On the basis of a unit factor difference between normal and rolled, a 1:2 ratio of pure and segregating families is expected. The two types of F_3 progenies occurred in exactly the expected proportions. Of the 48 progenies, 16 bred true for normal seedlings and 32 segregated for rolled. These data prove conclusively that rolled is a simple Mendelian recessive to the normal type of plant.

GENETIC INTERRELATIONS BETWEEN THE CHARACTERS

A virescent and W_1

An interesting relationship is exhibited between the virescent and white-base-leaf characters in the F_2 of crosses No. 1 and 2. Four types were found to occur in the proportion of 9 green:3 white-base-leaf:3 virescent:

1 albino, showing that the albino character is the result of an interaction between the factors v and w_l. A few families which show this segregation are arranged in table 1. The type called albino often developes a small amount of chlorophyll on the leaf tips, as well as other portions of the leaf. In some families, the classification between albino and white-base-leaf has been more or less arbitrary. It is a significant fact, however, that albino plants occur only in families segregating for V and W_l, never in monohybrid virescent or white-base-leaf families. The F_2 families from cross No. 1 are given in table 1 to show the interrelation between v and w_l because in those families the albino plants appear most distinct.

TABLE 1

F_2 segregation from plants having the composition $S_u S_u W_l w_l Vv$. Cross No. 1.

PEDIGREE NUMBER	GREEN	W. B. L.	VIRESCENT	ALBINO
212 (1)	161	62	44	15
863 (4)	104	25	36	12
(5)	125	37	28	10
(9)	125	51	41	11
(11)	195	69	75	17
(13)	115	39	37	17
(16)	121	48	32	18
(27)	153	55	52	15
864 (10)	170	63	51	24
(14)	161	55	42	18
(23)	48	20	25	5
Observed	1478	524	463	162
Calculated 9:3:3:1	*1477*	*493*	*493*	*164*

Deviation ÷ probable error (10:6) = 0.06

It will be noted in table 1 that the largest deviations occur in the two mean classes. This is probably due to the fact that virescent plants were erroneously classified as white-base-leaf, although the classification appeared fairly distinct. The excellent agreement of the calculated and observed ratios when the extreme and mean classes are grouped, indicates that an error was made in classifying the white-base-leaf and virescent types only. Further evidence that the albino character is determined by the double recessive condition $w_l v$ is furnished by grouping the non-albino types(green, white-base-leaf, and virescent) against the albinos in tables 1, 12 and 13. By such grouping the following F_2 ratio is found: non-albino seedlings 7837 (7818); albino seedlings 502 (521). It is very evident that this is a 15:1 ratio (Dev./P.E. = 19/14.92 = 1.27).

A virescent factor and Y

Several F_2 ears from cross No. 1 segregated for endosperm color, white-base-leaf seedlings, and virescent seedlings. It has been shown above that this particular virescent factor is independent of W_l and that the combination $v\,v\,w_l w_l$ produces an albino plant. Hence, the W_l factor may be eliminated and a chlorophyll ratio secured based on the segregation of the *V* factor only. In order to accomplish this the green plants and white-base-leaf plants belonging to each endosperm type were added together and designated as green plants. In like manner, virescent and albino plants were grouped and designated as virescents. The F_2 segregation secured by such grouping is as follows: yellow endosperm-green plants, 180 (179); yellow endosperm-virescent plants, 61 (59); white endosperm green plants, 56 (59); and white endosperm-virescent plants, 20 (20). The calculated ratio for independent inheritance is enclosed by parentheses. The observed ratio conforms very closely to the calculated, the deviation from a 10:6 ratio of independent inheritance being only 1.00 ± 5.81.

A virescent factor and C and R

The purple and colorless F_2 kernels from crosses No. 1 and No. 2 were planted separately and the segregates from each type of kernel noted. The F_2 results from mother ears of the composition *CRV.Crv* are as follows: purple kernels–green plants, 634 (625.5); purple kernels–virescent plants, 205 (208.5); colorless kernels–green plants, 197 (208.5); and colorless kernels–virescent plants 76 (69.5). For a 10:6 ratio the deviation over the probable error is 1.38. The close agreement of the observed ratio with the theoretical justifies the conclusion that *R* and *V* are independently inherited.

Further evidence that this *V* is independent of *R* and also of *C* is furnished by F_2 data from cross No. 2. The F_2 segregation from plants of the composition *cRV · Crv* is as follows: purple kernels—green plants, 123 (118); purple kernels—virescent plants, 33 (39); colorless kernels—green plants 95 (92); and colorless kernels—virescent plants, 28 (30). The close agreement of the observed ratio and that calculated for independent inheritance indicates that the factors *C*, *R*, and *V* are carried on separate chromosomes, the deviation over the probable error for a 34:30 ratio being only 0.53.

A greenish and light virescent

In the spring of 1923 a selfed ear J 45–7 of Four County, a selection from Silver King, was secured from Mr. M. T. JENKINS, of the IOWA

EXPERIMENT STATION at Ames. This ear was of interest because it contained two very distinct virescents which occurred in a good commercial variety of corn. The seed when planted in the greenhouse gave 145 green seedlings to 96 virescent seedlings, a 9:7 ratio with a deviation of 9 ± 5.19.

In chlorophyll development, the two virescents are very similar, the only difference being that one type develops considerably more chlorophyll than the other, although neither has been found to live beyond the three-leaf stage. The two characters have been designated as greenish-virescent and light-virescent respectively. Symbols are not given to the types at present because their linkage relations and identity with other known virescents have not been determined. In a dihybrid segregation these virescents grade into one another so that they cannot be accurately classified, but in monohybrid segregations the families containing greenish and light virescents can be easily distinguished.

TABLE 2

Progenies and their seedling segregations which resulted from self-pollinating green plants in a 9:7 virescent family (J45–7 selfed).

CHARACTER OF SEGREGATION	OBSERVED	CALCULATED	SEEDLING SEGREGATES		RATIO	DEV. / P. E.
			Green	Virescent		
Pure Green	1	*1.9*	95	..	..	..
Splitting for greenish virescent	3	*3.8*	566	171	3:1	1.64
Splitting for light virescent	3	*3.8*	542	183	3:1	.25
Splitting for both	10	*7.5*	1308	1002	9:7	.56

P for progeny test = 0.67

The ratio of the progenies and a summary of their segregations are arranged in table 2. In the progeny test, the closeness of fit (P), based on an independent assortment of the two virescent factors, is 0.67. The 9:7 green and virescent families likewise show a good agreement with the calculated ratio, assuming independent inheritance between the factors for greenish and light virescent. Here the deviation over the probable error is equal to .56.

Rolled and virescent

In the spring of 1923, Doctor LINDSTROM turned over to the writer two crosses which combined the characters: anther ear dwarf (a_n), virescent (v), sugary (s_u), and liguleless (l_g). A new character which had not hitherto been observed occurred in the F_2 generation of these crosses. It has been called rolled from the characteristic rolled condition of the

seedling leaf. The character evidently originated from the family to which the male parent belonged (L 4260). It seems most logical to believe this because the F_2 segregation of hybrids involving both L 4260–4 and L 4260–7 contained rolled plants, although they were crossed on two families which were unrelated to one another. Family L 4260 came originally from Doctor C. N. COLLINS.

The crosses involving rolled (r_o) may be represented factorially as follows:

Cross No. 3 L 4254–38 ($S_uS_u\ L_gl_g\ R_oR_o$) × L 4260–7 ($s_us_u\ L_gL_g\ R_or_o$)

Cross No. 4 L 4263–3 ($S_uS_u\ Vv\ A_na_n\ R_oR_o$) × L 4260–4 ($s_us_u\ VV\ A_nA_n\ R_or_o$)

In the second generation of cross No. 4, three families segregated for both rolled and virescent. A summary of these families is given in table 3.

TABLE 3

F_2 seedling segregation of rolled and virescent. Cross No. 5.

PEDIGREE NUMBERS	NORMAL SEEDLINGS		ROLLED SEEDLINGS	
	Green	Virescent	Green	Virescent
888 (3)	107	35	27	15
(9)	166	48	44	9
(20)	170	60	61	18
Observed	443	143	132	42
Calculated 9:3:3:1	*427*	*143*	*143*	*47*

Deviation ÷ probable error (10:6) = 1.22

If the factors for rolled and virescent were linked in inheritance, an excess of plants would be expected to occur in the mean F_2 classes. But a small excess occurs in the extreme classes when the ratio is calculated for independent inheritance and the mean classes grouped against the extreme classes. An equal deviation is expected to occur in 41 percent of the trials due only to errors of random sampling, the deviation over the probable error being equal to 1.22. Hence, it is safe to conclude that the factors R_o and V are inherited independently.

Liguleless and rolled.

Very little difficulty was experienced in classifying the rolled plants as to the ligule condition, provided only that the plants were noted before the leaves blackened and fell from the plant stem. It might be stated again, however, that only a small proportion of the rolled plants are lethal to this extent.

Three F_2 families from cross No. 3 segregated for liguleless and rolled. The data are given in table 4. The agreement of the observed ratio with the calculated, assuming independent inheritance between L_g and R_o, is

TABLE 4

Showing the F_2 families from Cross No. 4 which segregated for liguleless and rolled.

PEDIGREE NUMBER	NORMAL		ROLLED	
	Liguled	Liguleless	Liguled	Liguleless
889 (31)	63	23	22	6
(40)	72	24	20	11
L5527 (17)	121	44	35	11
Observed	256	91	77	28
Calculated 9:3:3:1	*254*	*85*	*85*	*28*

Deviation ÷ probable error (10:6) = 0.29

TABLE 5

Showing the F_2 families from Cross No. 4 which segregated for sugary and rolled.

PEDIGREE NUMBER	STARCHY KERNELS		SUGARY KERNELS	
	Normal	Rolled	Normal	Rolled
291 (4)	107	38	49	13
(7)	244	77	83	24
889 (1)	32	9	5	3
(8)	80	23	25	7
(28)	114	51	22	8
(31)	66	24	20	4
(35)	101	29	19	6
(36)	87	32	11	3
(40)	73	22	23	9
L5527 (1)	68	30	44	7
(2)	120	37	40	13
(13)	129	45	59	17
(16)	120	53	49	11
(17)	131	35	34	11
Observed	1472	505	483	136
Calculated 9:3:3:1	*1460*	*487*	*487*	*162*

Deviation ÷ probable error (10:6) = 0.84

very close. The deviation over the probable error based on a 10:6 ratio is only 0.29. These results prove that L_g and R_o are inherited independently.

Sugary and rolled

The F_2 progenies from cross No. 4 which segregated for sugary and rolled are given in table 5. The composition of the parent plants as regards

sugary and rolled may be represented factorially as $S_uR_o \cdot s_ur_o$. It will be noted in the table that the deviations from an independent segregation of the two characters are rather small, and that the mean classes are in excess of the expected numbers. If R_o and S_u were linked, the excess would have occurred in the extreme classes. When calculated for independent inheritance with a 10:6 grouping, the deviation is only 14 ± 16.6. It is very evident from these results that the factors for sugary and rolled are independent in inheritance.

Rolled and anther ear dwarf

The different F_2 classes in the segregation of rolled and anther ear dwarf, from cross No. 4, were fairly distinct. However, there was sometimes an uncertainty as to the classification of dwarf-rolled plants when they were observed in the early three-leaf stage. This was due to the fact that dwarf plants have shorter, broader and slightly thicker leaves than do normal plants, consequently the rolling of the leaves is less definite. The dwarf-rolled types could be identified with more certainty if the plants were allowed to grow four or five days longer than the tall rolled plants.

TABLE 6

F_2 seedling segregation of rolled and anther ear dwarf. Cross No. 4.

PEDIGREE NUMBER	NORMAL PLANTS		ROLLED PLANTS	
	Tall	Dwarf	Tall	Dwarf
888 (7)	174	51	50	13
(34)	75	22	26	6
(49)	74	24	30	12
Observed	321	97	106	31
Calculated 9:3:3:1	*312*	*104*	*104*	*35*

Deviation ÷ probable error (10:6) = 0.65

Three F_2 families which segregated for rolled and dwarf are given in table 6. The agreement of the observed and calculated ratios with independent inheritance is unusually close, the deviation from a 10:6 ratio being only 5 ± 7.7. Evidently the factors R_o and A_n are inherited independently.

LINKAGE RELATIONS

Factors V_6, V_7 and Y

The first linkage data involving the *Y* (yellow endosperm) factor and establishing the *Y* linkage group was reported by EMERSON (1921), *Y* being linked to P_l, the factor for purple plant color.

The reasons for designating the virescents reported herein as v_6 and v_7 are given in the discussion. In the spring of 1923 a cross was made between two virescent-carrying strains of commercial corn: J 136–5 from U.S. selection 133, a yellow dent corn, and J 121–4 from Silver King. The strains were planted under field numbers 232 and 230 respectively. The family 230 carried two very similar medium virescent types, one of which has been designated v_7. These two virescents are either independent or loosely linked as indicated by their linkage relations with *Y* and their F_2 green-virescent ratio. When V_7 alone was segregating, the crossing over percentage with *Y* was found to 41.5. When both virescent factors were segregating and the independence of the second virescent was assumed, a crossing over percentage of 35.5 was found. Chance alone could cause an equally large difference in the crossing over percentage. Again, when the green-virescent segregates of table 8 are calculated for independent inheritance a deficiency of 20 virescents was found, the deviation over the probable error being 2.23. The deficiency may have been caused in part by the difficulty in classifying the virescent and green plants. Further tests would be necessary to definitely determine the genetic relation of these two virescents.

The cross between a normal green plant from 232 and a pure virescent plant from 230 may be represented factorially as follows:

Cross No. 5 232–11 ($YY\ V_7V_7\ VV\ V_6v_6$)×230–1 ($yy\ v_7v_7\ Vv\ V_6V_6$)

The F_1 plants were all normal green, showing that different virescent factors were involved. Among seven F_2 progenies, two trihybrid green and virescent, and two dihybrid medium virescent segregating progenies occurred. One must conclude from this that the pure virescent plant 230–1 was heterozygous for a second virescent factor. The two trihybrid segregating families produced a total of 283 green seedlings to 329 virescents. The deficiency of virescents was caused largely by errors in the classification of some greenish virescents. Data from these families are not included in the linkage calculations.

One F_2 family, 921–9 segregated for one of the medium virescents, v_7. The relation of this virescent to yellow endosperm may be seen in table 7. From the composition of the parents, the coupling phase would be expected if *Y* and the virescent factor were linked. An excess of 47 plants did occur in the extreme classes when based on the calculation for independent inheritance, the deviation over the probable error having the value of 4.6. A gametic ratio of 1.41:1 was found, indicating coupling linkage between *Y* and V_7, with a crossing over percentage of 41.5. The

closeness of fit was fair, P being equal to 0.38. However, the difficulty in classifying some of the virescent plants lowered this value to some extent.

Two F_2 families segregated for the two medium virescents. The seedling counts from these families are given in table 8. Here again the coupling phase of linkage between the factors V and V_7 is exhibited. The percentage of crossing over was found to be 35.5 with a value for P of 0.40.

TABLE 7

F_2 segregation from plants of the composition $YV_7\ VV_8 \cdot yv_7\ VV_8$. Cross No. 5.

PEDIGREE NUMBER	YELLOW ENDOSPERM		WHITE ENDOSPERM	
	Green	Virescent	Green	Virescent
921 (9)	592	149	178	79
Observed	592	149	178	79
Calculated (1.41:1 linkage)	*585*	*164*	*164*	*85*

P=0.38

The weighted gametic ratio from the V and V_7 segregation shown in tables 7 and 8 is 1.61:1.00, which indicates an average crossing over percentage of 38.3.

TABLE 8

F_2 segregation from plants of the composition $YV_7\ VV_8 \cdot yv_7\ vV_8$. Cross No. 5.

PEDIGREE NUMBER	YELLOW ENDOSPERM		WHITE ENDOSPERM	
	Green	Virescent	Green	Virescent
921 (6)	203	130	46	44
(8)	242	147	60	72
Observed	445	277	106	116
Calculated (1.83:1 linkage)	*428*	*280*	*103*	*133*

P=0.40

Two other F_2 families segregated for light virescent (v_6) and medium virescent (v_7). These two families reveal a rather unusual occurrence. They furnish evidence that both virescent factors are borne on the same chromosome with the factor for yellow endosperm. The relation of the two virescents to one another may be seen in table 9. It will be noted that the inter-relation between medium virescent and light virescent is similar to that between the virescent and albino characters. The factor for light virescent dominates the factor for medium virescent, consequently

they would produce the ordinary 9:3:4 ratio if they were not linked in inheritance. There is an excess of 17 plants over the number expected with independent inheritance between the two virescent factors. This indicates repulsion linkage which is expected since the virescents entered the cross from different parents. A gametic ratio of 1:1.37, indicating a cross over percentage of 42.2 between V_6 and V_7 is shown by the F_2 data.

TABLE 9

F_2 segregation from plants of the composition $YV_7\ Vv_6 \cdot yv_7\ VV_6$. Cross No. 5.

PEDIGREE NUMBER	GREEN	MEDIUM VIRESCENT	LIGHT VIRESCENT
921 (1)	209	81	104
(7)	288	98	133
Observed	497	179	237
Grouping virescents	497	416	
Calculated :(1:1.37 linkage)	*497*	*416*	

Those families segregating for the factors V_6, V_7, and Y are given in table 10. Before calculating the linkage relations, the medium virescent plants were grouped with the green plants under both the yellow and white endosperm classes. This is legitimate because the factor for light

TABLE 10

F_2 segregation from plants of the composition $YV_7\ Vv_6 \cdot yv_7\ VV_6$, with classes grouped to show the relation between Y and V_6. Cross No. 5.

PEDIGREE NUMBER	YELLOW ENDOSPERM			WHITE ENDOSPERM		
	Green	Medium Virescent	Light Virescent	Green	Medium Virescent	Light Virescent
921 (1)	146	49	95	63	32	9
(7)	211	61	130	77	37	3
Observed	357	110	225	140	69	12
Grouping	467		225	209		12
Calculated (1:3.5 linkage)	*468*		*217*	*217*		*11*

$\chi^2 = 0.683$ P = very good fit

virescent expresses itself in the presence as well as in the absence of the factor for medium virescent. Hence, the light virescent plants occur in undisturbed proportions among the yellow and white endosperm classes. Linkage calculations revealed 23 percent of crossing over between Y and V_6. The closeness of fit is very good, the value of χ^2 being only 0.683.

A summary of the above linkage tests may be given in the form of a chromosome map as follows:

V_6.....23.....Y.....38.....V_7

.....42.....

The relation of V_6 and V_7 to the other factors in the linkage group has not been determined. Therefore, the true position of V_6 and V_7 in relation to Y may be reversed from that shown in the chromosome map. It is believed that a more distinct strain of the medium virescent material (v_7) should be isolated if possible before making outcrosses for linkage tests.

Relation between the factors W_l and S_u

STROMAN (1924b) has shown that the factor for white-base-leaf (w_l) and sugary endosperm (s_u) are linked with about 25 percent of crossing over. The writer has accumulated additional data verifying the linkage of W_l and S_u.

During the summer of 1922 crosses were made in order to study the interrelation of kernel characters and the white-base-leaf character.

The crosses may be represented factorially as follows:

Cross No. 1, L 4992–23×S 899–7

AA Cc Rr $S_u\ s_u$ *Yy* $W_l\ W_l$ *VV* $S_c\ S_c$ ×

AA Cc rr $S_u\ S_u$ *YY* $w_l\ w_l$ *Vv* $S_c\ s_c$

Cross No. 2, L 4992–26×S 899–10

AA Cc Rr $S_u\ s_u$ $W_l\ W_l$ *VV* ×

AA CC rr $S_u\ S_u$ $w_l\ w_l$ *Vv*

Family L 4992 was the F_1 of a cross between Black Mexican sweet corn and Golden Glow, a yellow dent corn. Family S 899 was a homozygous F_3 white-base-leaf family, originating from a hybrid ear (A 1222–3×A 1315–8) made by Dr. G. N. STROMAN from material secured from Dr. E. G. ANDERSON. Ear 1222–3 was factorially *A A C c* $s_u\ s_u$. The other ear, A 1315–8, came from CASTLE's number 123, being heterozygous for some chlorophyll deficiencies and defective seed.

In arranging the F_2 and F_3 data from crosses No. 1 and No. 2, those families which segregated for sugary and white-base-leaf only ($S_u\ s_u$ $W_l\ w_l$ *V V*) were placed in separate groups from those families which segregated for virescent in addition ($S_u\ s_u$ $W_l\ w_l$ *V v*).

The degrees of linkage in the simple dihybrid segregations were calculated by the method described by EMERSON (1916). For those families which segregated for sugary, white-base-leaf, and virescent types, the

green plants were grouped against the non-greens, the latter class including the characters white-base-leaf, virescent and albino. The gametic ratio was then calculated by formulae suggested by BRUNSON (1924) for use with trihybrid ratios involving two complementary genes, one of which is linked to a third gene. In the present case the genes for virescent and white-base-leaf are the two complementary genes, the factor for white-base-leaf being the complementary gene which is linked to a third gene, the factor for sugary.

Those F_2 families which showed the dihybrid segregation for sugary and white-base-leaf are given in table 11. The calculated gametic ratio was found to be 1:3.2. It will be seen in the table that there is an excess

TABLE 11

F_2 segregation from plants having the composition S_u w_l V · s_u W_l V. Crosses 1 and 2.

PEDIGREE NUMBER	STARCHY		SUGARY	
	Green	W. B. L.	Green	W. B. L.
211 (10)	179	85	51	2
863 (17)	110	50	51	2
(24)	113	71	50	5
(26)	193	74	83	3
864 (17)	125	78	61	0
865 (1)	135	78	68	0
(8)	175	80	79	5
(14)	176	66	78	2
(23)	115	44	45	2
(28)	108	53	60	3
866 (4)	150	41	87	0
(9)	66	43	33	1
(10)	83	31	26	1
(12)	176	66	95	5
(22)	93	46	37	1
Observed	1997	906	904	32
Calculated (1:3.2 linkage)	*1974*	*905*	*905*	*55*

P = 0.02

of plants occurring in the starchy green class and a deficiency in the sugary white-base-leaf class. The deficiency of white-base-leaf plants among the sugary kernels is very significant because of the smallness of the class resulting from repulsion linkage. The deviations in the other classes are negligible. These points may be illustrated by the closeness of fit calculations for table 11 as follows:

Phenotypes	Observed	Calculated, 1:3.2 linkage	(0–C)	(0–C)²	(0–C)²/C
Starchy green	1997	1974	23	529	0.268
Starchy white-base-leaf	906	905	1	1	0.001
Sugary green	904	905	1	1	0.001
Sugary white-base-leaf	32	55	23	529	9.618
				χ^2=	9.888
Closeness of fit for total				P=	0.02

This does not explain the deviations, however. The excess of plants in the double dominant class might be accounted for on the basis of pure chance variation or contamination, but it is harder to account for the large proportional minus deviation in the double recessive class. The deficiency may be due partly to the poor germination of the sugary kernels

TABLE 12

F_2 segregation from plants having the composition $S_u\ w_l v \cdot s_u\ W_l V$. Crosses No. 1 and 2.

PEDIGREE NUMBER	STARCHY KERNELS				SUGARY KERNELS			
	Gr.	W. B. L.	Vir.	Alb.	Gr.	W. B. L.	Vir.	Alb.
209 (8)	137	69	43	31	62	0	18	0
211 (8)	12	8	2	2	5	0	0	0
(9)	97	54	42	15	46	1	13	1
863 (2)	66	40	26	16	43	3	14	0
(3)	72	23	25	12	30	1	13	0
(6)	128	58	48	19	49	5	12	3
(14)	130	54	41	18	66	2	15	2
(31)	91	43	39	15	35	4	8	0
864 (20)	103	49	40	11	54	3	13	2
865 (4)	79	50	20	7	42	5	2	0
(6)	67	42	24	8	32	2	18	0
(7)	64	31	14	11	30	1	11	0
(20)	79	34	24	13	34	1	7	0
(24)	90	48	39	15	42	3	8	0
(32)	70	33	30	8	28	3	10	1
(33)	88	52	31	10	45	2	10	1
866 (5)	84	36	22	11	34	3	12	1
(7)	70	29	26	10	29	0	14	1
(20)	41	13	9	3	18	2	3	2
(27)	77	33	23	6	36	0	14	1
(40)	133	53	49	19	51	4	31	1
Observed	1778	852	617	260	811	45	246	16
Grouping	1778		1729		811		307	
Calculated 1:4.3 linkage	*1765*		*1704*		*836*		*320*	

P=0.63

in the double recessive class, the germination of sugary kernels being 4 percent lower than that of the starchy. Unfortunately, double recessive plants were not available for backcrossing to F_1 hybrids during the summer of 1924.

TABLE 13

Summary of the F_2 progeny test from crosses No. 1 and 2.

NUMBER OF FAMILIES INCLUDED	STARCHY KERNELS				SUGARY KERNELS			
	Green	W. B. L.	Vir.	Alb.	Green	W. B. L.	Vir.	Alb.
5	382	121						
Calculated (3:1)	*377*	*126*						
Deviation ÷ probable error (3:1) = 0.76								
6	258	89	88	25				
Calculated (9:3:3:1)	*259*	*86*	*86*	*29*				
Deviation ÷ probable error (10:6) = 0.71								
2	304				78			
1	74		28		20		8	
Calculated (9:3:3:1)	*73*		*24.5*		*24.5*		*8*	
Deviation ÷ probable error (10:6) = 0.27								
11	831	382			336	16		
Calculated (1:2.48 linkage)	*815*	*359*			*359*	*32*		
P = 0.01								
5	267	118	78	38	91	7	27	1
Grouping	267		234		91		35	
Calculated (1:1.9 linkage)	*249*		*221*		*104*		*53*	
P = 0.02								
1	130	9			12	21		
Calculated (6.62:1 linkage)	*118*	*10.5*			*10.5*	32		
P = .15								
1					12			3
7					799			

Those F_2 families which segregated for sugary, white-base-leaf, and virescent are given in table 12. Again it will be noted that there is an excess of plants over the calculated among the starchy classes and a deficiency among the sugary classes. The linkage calculations show a crossing over percentage of 18.9 between W_l and S_u. A value for P of 0.63 is shown, which is much higher than that of the former table. The method of grouping is partly responsible for the higher value of P.

An F_3 progeny test of white-base-leaf and sugary including 38 families is shown in table 13. Of these families, sixteen exhibited the repulsion phase of linkage between W_l and S_u and one exhibited the coupling phase. The 16 families were grouped into two classes, those not segregating for virescent and those segregating for it. Two groups thus classified showed repulsion linkage. The one family which exhibited linkage in the coupling phase did not segregate for virescent. The crossover percentages given by the three groups was 28.7, 34.5, and 13.1 respectively. The latter figure is based on only 172 plants. In the F_3 generation, the closeness of fit is similar to that of F_2 in being very low (as shown in table 13), the values of P for the three sets of data being 0.01, 0.02, and 0.15.

TABLE 14

Summary of F_2 genotypes as indicated by the F_3 progeny test given in table 13.

F_2 GENOTYPES AND CALCULATED RATIOS (1:4 LINKAGE)	OBSERVED NUMBER	CALCULATED NUMBERS (1:4 LINKAGE)	DEVIATIONS
1 $S_u S_u$ $W_l W_l$	0	.51	.51
8 $S_u S_u$ $W_l w_l$	11	4.06	6.94
8 $S_u s_u$ $W_l W_l$	2	4.06	2.06
2 S_u $W_l s_u w_l$	1	1.01	.01
32 S_u $w_l s_u W_l$	16	16.21	.21
.. $S_u w_l$ (no selfs)	..	..	..
16 $s_u s_u$ $W_l W_l$	8	8.10	.10
8 $s_u s_u$ $W_l w_l$	0	4.05	4.05
.. $s_u w_l$ (no selfs)	..	..	..
Total	38	38.00	

P=0.008

A summary of the F_2 genotypes is given in table 14. It will be noted that the progeny test shows a very aberrant ratio. The F_2 genotypes occur in such fashion that it would be impractical to calculate the percentage of crossing over by the ordinary r and s method. When the calculation is based only on the number of families showing the repulsion linkage (16) and the coupling linkage (1), a crossing over percentage of 20 is found. On account of the great deviations in the other F_2 genotypes, the percent

of crossing over has little meaning. It will be noted that the largest deviations occur in the $S_u S_u \; W_l w_l$ and $s_u s_u \; W_l w_l$ classes, seven progenies more than expected occurring in the former class, and none occurring in the latter class where four were expected. Differential viability has no doubt played some part in causing the deviations, for there were many F_2 plants which did not produce an ear. Another explanation for the aberrant F_2 and F_3 seedling ratios may be that the w_l factor is unable to express itself in the presence of s_u under some conditions. It must be admitted that more study will be necessary before the S_u and W_l relationship can be satisfactorily explained.

TABLE 15

Showing the summary of fifteen F_2 families as regards scarred endosperm and sectored plants. Cross No. 1.

PEDIGREE NUMBER	KERNELS		PLANTS FROM NORMAL KERNELS		PLANTS FROM SCARRED KERNELS	
	Normal	Scarred	Non-sectored	Sectored	Non-sectored	Sectored
Observed, fifteen ears segregating for scarred endosperm	3906	298	3694	2	68	97
Calculated (15:1)	3941	263				
Deviation ÷ probable error (15:1) = 3.30			Percent of sectored plants .05		Percent of sectored plants 58.8	

The average weighted gametic ratio of all the F_2 and F_3 families was found to be 1:3.54 which indicates a crossing over percentage of 22 between the factors W_l and S_u. It is not known what weight should be placed on the crossover percentage (20) calculated from the F_3 progeny test. From the data given herein it seems safe to conclude that the factors S_u and W_l are about 20 or 22 units apart.

Relation between scarred endosperm (s_c) and sectored plants

A very definite correlation between sectored plants and scarred endosperm kernels was shown in the second generation from cross No. 1. The intensity of scarring on the endosperm is apparently not associated with a relative degree of striping on the sectored plants, because intensely scarred kernels often produced normal green plants. Table 15 shows the summary of all the counts which were made from ears segregating for scarred endosperm. The occurrence of the two sectored plants among normal kernels might well be the result of errors in the classification of the kernels. Due to the difficulty in classifying the scarred kernels, and to

the variation in the sectoring of the plants, it is not possible to determine the exact genetic relation of the two characters at present. However, the occurrence of 59 percent of sectored plants among scarred endosperm kernels and of only .05 percent among the normal kernels indicates a very close relationship between the two characters.

DISCUSSION

In this paper is reported the linkage of two virescent factors V_6 and V_7 lying about 23 and 38 units respectively from the factor Y. Evidence shall here be given for believing that these virescent factors are genetically different from the factors V_1, V_2, V_3, V_4, and V_5 reported by DEMEREC (1924). He found that V_1 belongs to the C–W_z linkage group, and V_4 to the B–L_g group; therefore neither V_1 nor V_4 can be linked with Y to which the present genes are linked. He has also found (unpublished data) that V_2, which is probably identical with STROMAN'S V_1 (1924a), is not linked to Y. Other data, while not entirely conclusive, furnish a rather strong indication that the factors V_3 and Y are likewise independent. His counts are as follows: yellow endosperm—green seedlings, 95 (96); yellow endosperm—virescent seedlings 29 (32); white endosperm—green seedlings 35 (32); white endosperm—virescent seedlings 11 (10). The theoretical ratio for independent inheritance is placed in parentheses. When the ratio is grouped on a 10:6 basis, the deviation is zero. Hence, it seems safe to conclude that the factor V_3 is genetically different from the writer's virescent types in that the distances of the latter from Y are about 23 and 38 units. The virescent factor V_5 was found by KVAKAN (1924) to belong to the linkage group with brown aleurone (B_n), glossy (g_l), and ramosa (r_a). Hence, it is also independent of Y. It appears, therefore, that the evidence in favor of the two present virescent genes being different from those heretofore reported is sufficient to justify assigning them the next two higher numerals in the virescent series, as V_6 and V_7.

The yellow virescent factor which was carried by the male parent of crosses No. 1 and 2 is independent of Y. It is therefore independent of and genetically different from V_6 and V_7. A third virescent of cross No. 5 associated with V_7 is either independent of or loosely linked to V_7.

The two virescent types originating from a selfed ear of the variety Four County White proved to be independent of one another. Their relation to the other virescents reported herein has not been tested. The same is true in cross No. 4 of the virescent which was found to be independent of rolled seedlings, anther ear dwarf, and sugary.

In the second generation of crosses No. 3 and No. 4 there appeared a semi-lethal seedling character which has been called rolled. It answers very closely to the description of the disease scutellum rot given by HOLBERT, et al (1924). Seed from a segregating ear was sent to Mr. HOLBERT for identification. He found it to be phenotypically different from scutellum rot. Contrary to prediction, this diseased appearing condition of the seedling leaves, kernel, and roots behaved as a simple Mendelian recessive through a second and third generation.

The interaction between different chlorophyll characters in maize furnishes an interesting genetic study. The types most deficient in chlorophyll ordinarily dominate the less deficient types when their factors occur in the double recessive condition. It has been further demonstrated by DEMEREC (1924) that the phenotype of seedlings recessive for two virescent factors is lighter in color than either of the parent types. The F_2 segregation of certain virescents gave the ratio, 9 green:6 virescent:1 very light virescent. A similar ratio was given by the characters fine-striped and a virescent. One rather similar case of the interaction between two chlorophyll types is reported in the present paper. This occurs in the case of white-base-leaf and the virescent of cross No. 1. The double recessive condition of the factors for these types produces an albino plant. The double recessive type, however, often develops a small amount of chlorophyll on its leaf tips. In some families it produces slightly more chlorophyll than any of the types commonly known as pure white (*w w*).

There is a similarity between virescents and albinos with respect to their number and linkage relations as reported by different geneticists. Seven different genes for albino seedlings and indications for many more have been found (DEMEREC 1923). Four albino genes occur in two linkage groups, while six different virescent factors occur in five linkage groups. In the *Y* group three factors for albino and two for virescent have been found.

Chlorophyll is extremely variable in its expression, for example, in maize the phenotype of chlorophyll varies from an almost entire absence of green through numerous shades and patters of greening to the full dark green color of our common commercial varieties. The large number of chlorophyll defects found in maize indicates the relative instability of the genes which determine chlorophyll, or else it indicates the presence of a very large number of such genes only a small percentage of which have mutated. However this may be, there is a close correspondence between the chemical complexity of chlorophyll and the number and diversity of the known chlorophyll mutants.

It seems that since chlorophyll is such a fundamentally important substance, nature has acted wisely in having not a few genes but many genes control it. Such an arrangement affords the greatest possible flexibility allowing ready adjustment to a changing environment.

SUMMARY

A virescent seedling type of maize having a distinct carotinoid-like appearance proved to be inherited independently of the factors *Y*, *C*, *R*, and W_l.

The factor for the above virescent and for white-base-leaf (w_l) produced in the F_2 generation a ratio of 9 green seedlings, 3 white-base-leaf seedlings, 3 virescent seedlings, and 1 albino seedling, the albino being of the genotype $w_l\ w_l\ v\ v$.

The seedling type, rolled (r_o), an abnormality of the leaves and underground parts, was found to be strictly Mendelian in inheritance.

Data are presented to show that the factor for rolled (r_o) is independent of the factors A_n, S_u, L_g, and one virescent factor.

Two virescents designated as v_6 and v_7 were found to be linked to *Y* with a crossing over percentage of 23 and 38 respectively. A crossing over percentage between v_6 and v_7 of about 42 was found, indicating that the two virescent factors are located on opposite ends of the chromosome.

Further evidence was found in support of a linkage between the factors S_u and W_l. The data, while highly variable, indicate between 20 and 22 percent of crossing over.

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INHERITANCE STUDIES IN SOYBEANS. I. COTYLEDON COLOR[1]

F. V. OWEN

Maine Agricultural Experiment Station, Orono, Maine.

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INTRODUCTION

TERAO (1918) and PIPER and MORSE (1923) have given evidence for maternal inheritance of cotyledon color in the soybean (*Soja max*). These authors assumed that yellow and green cotyledons were inherited strictly through the maternal parent because the same F_2 progenies segregated for other characters but not for cotyledon color. WOODWORTH (1921), however, was able to explain his results with cotyledon color by the use of Mendelian factors. The dominant factors *D* and *I* were equally capable of producing yellow cotyledons, so both 15:1 and 3:1 ratios were secured in F_3 progenies.

While engaged in a study of inheritance in the soybean the present writer has been able to confirm both types of inheritance. Conclusive proof has been obtained for the duplicate factors described by WOODWORTH and the case which appears to be maternal inheritance, although handicapped by more difficulties, seems very confirmative. More intercrosses would be very desirable to thoroughly establish the relationships between various varieties but a report at this time seems advisable since it is unlikely that the work can be continued.

DISTINCTION BETWEEN YELLOW AND GREEN COTYLEDONS

The cotyledons of all varieties of soybeans are green previous to maturity but in certain varieties the chlorophyll color fades out, or rather

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turns yellow, as the seed ripens. Light also has a very marked effect on this behavior because if seed with green cotyledons is exposed to strong sunlight even after it is fully ripe the color gradually fades out. The effect of pod color is therefore quite noticeable. Black-podded varieties with green cotyledons may produce normal green cotyledon color but it is very likely that the black pigment in the pods acts as a protective agency against the sun's rays. In F_2 segregates from crosses with varieties having yellow cotyledons, seeds with green cotyledons were sometimes borne on plants with light straw-colored pods. The plants that were studied remained in the field for some time after ripening and the green-cotyledon and green seed-coat colors were oftentimes badly faded out when the pods happened to be of the lighter color. With a knowledge of this environmental effect, however, the seeds from plants segregating for cotyledon color could usually be classified without great difficulty.

Perhaps the best time to distinguish the cotyledon colors is just before maturity, providing the seeds have an opportunity to become fully developed. At this stage green cotyledons are a very bright green and the yellow color is a distinct contrast. If normal maturity is interfered with some cotyledons appear green which are potentially yellow, but the writer was fortunate in having early maturing varieties, so there was very little difficulty on this account. It was interesting to note that seeds from varieties with yellow cotyledons, shelled when about half developed, remained green. On the other hand, seeds picked at the same time but allowed to remain in their pods became nearly as yellow as normally ripened seed. Shelling and drying the seeds, it seemed, interfered with the physiological processes which cause the cotyledon color to fade out at maturity.

EXPERIMENTAL WORK AND RESULTS

When the work began in 1923 two varieties with green cotyledons were grown, namely, the Medium Green or Guelph variety and an unidentified Chinese variety given the arbitrary number C4 in our records. A number of selections without a definite record were also grown but these all resembled the Medium Green variety very much, from which they undoubtedly originated. A large number of crosses proved successful with the selection No. C4 but in spite of a great deal of effort the opposite was true for the Medium Green variety. All the trials with the Medium Green variety as the maternal parent were failures and only four crosses with the Medium Green as the paternal parent were successful. (A cross (No. 71) was recorded with the Medium Green variety as the maternal

parent, but there are reasons to believe that a mistake was made on the original record, and that the order of the parents should be reversed.)

Duplicate factors for yellow cotyledons, crosses with selection No. C4

The genetic constitution of selection No. C4 so far as it is known is interpreted as follows, $LLTTGGI^{i}I^{i}R_1R_1d_1d_1d_2d_2$. Proof for all these factors will be given in Part III of this series. The only factors of interest

TABLE 1

Cotyledon color in F_2 generation. Crosses with green No. C4.

PARENTAL VARIETY WITH YELLOW COTYLEDONS	PROGENY NUMBERS	YELLOW COTYLEDONS	GREEN COTYLEDONS	DEV.	P. E.	DEV. / P. E.	ACTUAL RATIO
Mandarin	*(Mixed)	510	32	1.87	3.80	0.49	15.9:1
	18	335	26	3.44	3.10	1.11	12.9:1
	21	261	22	4.31	2.75	1.57	11.9:1
	23	275	12	5.94	2.77	2.14	22.9:1
	47	305	26	5.31	2.97	1.79	11.7:1
Total		1686	118	5.25	6.93	0.76	14.3:1
Aksarben	*(Mixed)	101	10	3.06	1.71	1.79	10.1:1
	72	358	22	1.75	3.18	0.55	16.3:1
	73	179	8	3.69	2.24	1.65	22.4:1
	74	420	26	1.87	3.45	0.54	16.1:1
	75	296	18	1.62	2.89	0.56	16.4:1
	76	422	29	0.81	3.47	0.23	14.6:1
	77	476	37	4.94	3.70	1.33	12.9:1
Total		2252	150	0.12	8.00	0.01	15.0:1
Ito San	51	35	2	0.31	0.54	0.57	17.5:1
	56	442	26	3.25	3.53	0.92	17.0:1
Total		477	28	3.56	3.67	0.97	17.0:1
Japanese glabrous variety No. (J1)	100	198	13	0.19	2.37	0.08	15.2:1
	101	190	11	1.56	2.31	0.68	17.3:1
Total		388	24	1.75	3.31	0.53	16.2:1
Manchu	58	93	10	3.56	1.66	2.14	9.3:1
Green No. C1	61	169	9	2.12	2.18	0.97	18.8:1
Grand Total		5065	339	1.25	12.00	0.10	14.9:1

* Seeds from plants grown in greenhouse.

at this time are G, d_1, and d_2. G (NAGAI, 1921) is synonymous with WOODWORTH'S (1921) factor V, and is the dominant factor for green plastid color in the seed coat. Factors d_1, and d_2 are synonymous with WOODWORTH'S factors d and i. They are recessive to D_1 or D_2 that is D_1 and D_2 are duplicates. All crosses between No. C4 and varieties with yellow cotyledons have proved the presence of these duplicate factors for cotyledon color. Table 1 gives the results in F_2 which show a very close fit to a 15:1 ratio. WOODWORTH (1921) reports very similar results for a similar cross but with less conclusive results in the F_2 generation.

It is also of interest that a linkage was found between one of the factors for cotyledon color and G, the factor for green color in the seed coat. A comparison is given in table 2 of the cotyledon color in the F_2 seeds and the seed-coat color of the plants grown from these seeds. Considering the total of all crosses, $\chi^2=10.37$ and $P=0.02$, if we consider cotyledon color inherited independently from seed-coat color.

TABLE 2

Cotyledon color of F_2 seeds compared with seed-coat color of F_2 plants.

PARENTAL VARIETY WITH YELLOW COTYLEDONS		PROGENY NUMBER	YELLOW COTYLEDONS		GREEN COTYLEDONS		
			Green seed coat	Yellow seed coat	Green seed coat	Yellow seed coat	
	Observed	13	236	85	16	..	On basis of independence $\chi^2=7.433$ P= 0.06
	Corrected figures[1]		232	84	21	..	
	Calculated on basis of 12.5 percent crossing over		*232.0*	*83.9*	*20.7*	*0.3*	
Aksarben	Observed	31	37	8	1	..	
Ito San	Observed	51	31	14	4	..	
Manchu	Observed	58	47	12	6	..	
Total	Observed		351	119	27	..	
	Corrected figures		348	118	31	..	$\chi^2=0.875$ P= 0.80
	Calculated on basis of 12.5 percent crossing-over		*342.2*	*123.8*	*30.5*	*0.5*	
	Corrected figures		348	118	31		$\chi^2=10.369$
	Calculated on basis of 45:15:3:1 ratio		*349.4*	*116.5*	*23.3*	*7.8*	P= 0.02
	Difference		−1.4	+1.5	+7.7	−7.8	

[1] WOODWORTH (1923) refers to EMERSON'S method of making these corrections. It seems perfectly logical because no effort was made to raise exactly 15 plants from seeds with yellow cotyledons to every plant that came from a seed with green cotyledons; yet there is good reason to believe that this is the ratio to be expected.

The most logical explanation is the same as that which WOODWORTH used in interpreting his results. If there are two factors for cotyledon color (D_1 and D_2), then G must be linked with one of these factors. Let us designate this D_1. Making this assumption, the formula, suggested by Collins (1924), $P=\sqrt{\frac{8\,(AB+ab)}{n}}-5.5$ should give the crossover value but since we are dealing with a case of strong repulsion an actual comparison with theoretical ratios seems better. (In this formula P represents the proportion of AB and ab gametes and $1-P$ represents the proportion of Ab and aB gametes.) From a comparison with the ratios which WOODWORTH (1923) has worked out it is seen that a gametic ratio of 1:7 gives a very good fit. The crossover percentage should, therefore, be approximately 12.5 percent. WOODWORTH postulated 13 percent crossing over so it is seen that the results are in very close agreement.

TABLE 3

Relation between cotyledon color in F_3 and seed-coat color in F_2 with duplicate factors for cotyledon color

TYPE	PROGENY NO. 13 (GREEN NO. C4× MANDARINS)				TOTAL FOR ALL PROGENIES OF CROSSES WITH GREEN NO. C4			
	Observed	Corrected figures	*Calculated on basis of 12.5 percent crossing over*	Deviation	Observed	Corrected figures	*Calculated on basis of 12.5 percent crossing over*	Deviation
a. All yellow cotyledon, green seed-coat.....	76	74.8	*78.0*	−3.2	116	115.0	*115.0*	0.0
b. All yellow cotyledon, yellow seed-coat....	71	69.9	*69.4*	+0.5	99	98.2	*102.4*	−4.2
c. 15 yellow:1 green cotyledon, green seed coat	72	70.9	*75.0*	−4.1	113	112.0	*110.7*	+1.3
d. 15 yellow:1 green cotyledon, yellow seed coat	11	10.8	*9.2*	+1.6	16	15.9	*13.6*	+2.3
e. 3 yellow:1 green cotyledon, green seed-coat	88	86.6	*79.0*	+7.6	122	121.0	*116.5*	+4.5
f. 3 yellow:1 green cotyledon, yellow seed-coat	3	3.0	*5.3*	−2.3	4	4.0	*7.8*	−3.8
g. All green cotyledon, green seed-coat.....	16	21.1	*20.7*	+0.3	27	31.0	*30.5*	+0.5
h. All green cotyledon, yellow seed-coat....			*0.3*	−0.3			*0.5*	−0.5
	$\chi^2=2.432$ P=0.93				$\chi^2=3.110$ P=0.87			

WOODWORTH's method may also be repeated in comparing the cotyledon color of the F_3 seeds with the seed-coat color of the F_2 plants. Table 3

gives this comparison. Again a formula, $P = \sqrt{\frac{16(a+c+e)}{n}} - 11$ (see table 3 for meaning of a, c and e) should give a close approximation for the crossover percentage if we were not dealing with strong repulsion where a very slight deviation in the ratios makes a large difference in the calculated percentage of crossing over. An actual comparison with theoretical ratios is much better and by making such a comparison a gametic ratio of 1:7 explains the results very well. It is safe to conclude, therefore, that G is linked to D_1 and that the crossover value is approximately 13 percent.

Maternal inheritance, crosses with selection No. 56 and No. 29.

It is very unfortunate that more crosses were not made with this variety because the limited amount of evidence that we do have indicates that there is a different situation from that reported in crosses with No. C4.

Two crosses were made with progeny No. 56, which is a selection from the Medium Green variety. One cross was with a Mandarin and the other was with an Aksarben. To make the situation still more unfortunate the F_1 plants were grown in the greenhouse (1924–25) and it so happened that they were included among the plants which did very poorly. Only 19 seeds were borne on the two plants.

In 1925 the F_2 seeds were planted and 16 plants grew from the Mandarin × Medium Green cross and 2 from the Aksarben × Medium Green cross. These plants segregated for color of pubescence, seed-coat color, and flower color, but all the cotyledons remained yellow. It is highly improbable that there could have been a segregation according to a 15:1 ratio in the F_2 seeds because if such had been the case only 7/16 of the F_2 plants should have produced seed with all cotyledons yellow. The odds against this being due to chance are over two million to one, so duplicate factors could hardly have been responsible for cotyledon color in this case. Breeding results could be explained if a series of independent factors had the same effect as D_1 and D_2 in the preceding case, thus decreasing the probability of securing an individual recessive for all factors. If this cannot be proved true maternal inheritance is the only remaining explanation.

A very similar case of inheritance was also observed in a cross between a Medium Green selection (No. 29) and a Manchu selection (No. SP 3–9). On the F_1 plant 250 F_2 seeds were borne all of which had yellow cotyledons. Furthermore, 86 F_2 plants were grown from this seed and all F_3 seed had yellow cotyledons. Maternal inheritance seems the most

logical explanation but according to all records the Medium Green parent with green cotyledons was the maternal parent rather than the paternal parent as this theory presupposes. It seems very likely, however, that the order of the parents in the original cross was reversed.

DISCUSSION AND CONCLUSIONS

Two duplicate factors D_1 and D_2 have been described which are responsible for yellow cotyledons in soybeans. One factor designated as D_1 is linked with G the factor for green seed-coat color (NAGAI 1921), giving approximately 13 percent crossing over. These findings confirm WOODWORTH'S (1921) observations and D_1 and D_2 have been identified in the Mandarin, Aksarben, Ito San, and Manchu varieties besides two other unidentified selections.

Although the duplicate factors are very well established other modes of inheritance must be assumed to explain the results of crosses with the Medium Green variety. Maternal inheritance seems the most logical explanation and this confirms the findings of TERAO (1918) and PIPER and MORSE (1923) but a repetition of these crosses would be very desirable because clear cases of this type of inheritance are not commonly reported. Maternal inheritance in other plants have been described in Malandrium, Antirrhinum, Aquilegia (BAUR 1909), Mirabilis, Urtica, Lunaria (CORRENS 1909), Melandrium (SHULL 1914), Primula (GREGORY 1915), Pisum (KAJANUS 1923) and Zea (ANDERSON 1923). All these cases of maternal inheritance are concerned in some way with chlorophyll deficiencies. The green cotyledon color in soybeans, although also a chromatophore character (TERAO 1918), seems the most "normal" character thus far reported to have only a maternal origin.

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MENDELIAN INHERITANCE OF CHROMOSOME SHAPE IN MATTHIOLA[1]

MARGARET MANN LESLEY AND HOWARD B. FROST[2]

University of California, Citrus Experiment Station, Riverside, California.

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INTRODUCTION

A variety of stock (*Matthiola incana* R. Br.) known as Snowflake has been shown by FROST (1919) to produce frequent mutants of at least eight distinct kinds. These mutant forms are genetically inconstant, part of the progeny always being normals. These forms have been determined (FROST and MANN 1924, and papers in preparation) to be trisomic. Within the variety Snowflake, all plants cytologically examined, whether normal or with one or more extra chromosomes, have had strikingly long first-metaphase chromosomes in their pollen mother cells.

Miss ALLEN (1924) figured chromosomes for several pure single varieties of Matthiola, showing them to be much shorter than those found in Snowflake. The four pure single races used in the hybridization studies described below likewise all had very short chromosomes at and immediately before the first metaphase. The contrast in chromosome shape between Snowflake and these pure single varieties was very striking.

These short-chromosome varieties were crossed with Snowflake trisomics. The F_1 hybrids had short chromosomes, about like those of the pure single parents. It had seemed possible that the first-metaphase bivalents of these hybrids might each consist of a short and a long element, representing the respective parental types, but no suggestion of such a situation was observed. The short form was dominant in the group as a whole; each bivalent was composed of two similar members, whose source could not be determined. When F_2 hybrids were examined, it was at once evident that genetic segregation for chromosome shape had oc-

[1] Paper No. 160, UNIVERSITY OF CALIFORNIA, GRADUATE SCHOOL OF TROPICAL AGRICULTURE and CITRUS EXPERIMENT STATION, Riverside, California.

[2] Except as noted below, the cytological evidence presented in this paper has been secured by Mrs. LESLEY (Division of Genetics, UNIVERSITY OF CALIFORNIA), who has prepared all the figures. The breeding work has been done and the evidence on somatic characters secured by FROST (CITRUS EXPERIMENT STATION), who also made some of the tabulated determinations of chromosome shape, including the earlier ones made for the short-chromosome races and their F_1 hybrids.

curred. Every plant clearly belonged to one of the P_1 types, and the F_2 ratio indicated monohybrid segregation.

The pollen mother cells were studied in smears stained in Belling's iron-aceto-carmine. The roots were fixed in chrom-acetic-urea and stained in iron-haematoxylin. The material for the special examination of tetrads was all collected in May, 1926. Some rather marked variations in the apparent bulk of the meiotic chromosomes in the figures are to be attributed to variable swelling in acetic acid. This must probably be attributed to differences in the condition of the chromatin, since differences quite as great are often found in the same smear.

Since the work on chromosome shape has been incidental to study of trisomy and tetrasomy, the F_1 and F_2 data represent mainly plants with extra chromosomes, and the F_2 plants, with the exception of 6 of the 19 normals, were progeny of trisomic F_1 individuals. It is probable that the ratio for chromosome shape was not affected greatly if at all by trisomy, however, for the following reasons: (1) The extra chromosome of the F_1 plants was presumably derived in most if not all cases from the variety (Snowflake) that carried the recessive gene for chromosome shape; (2) in at least three of the trisomic types (Large, Slender and Small), the extra chromosome is a fragment which usually segregates at meiosis, in the pollen mother cells, independently of the normal pair from which it was derived; (3) the various trisomic F_1 parental types gave the following results: Small parent, 20 progeny, ratio 14:6; Slender parents, 12 progeny, ratio 9:3; Smooth parents, 12 progeny, ratio 9:3; other parents (five types), 18 progeny, ratio 13:5.

THE PARENTAL RACES AND THEIR F_1 HYBRIDS

Snowflake is a glabrous, white-flowered, double-throwing variety, medium early in blooming and of rather open growth habit. Two of the pure single races were grown from seed kindly furnished by Miss EDITH R. SAUNDERS. Both of these are glabrous, rather small, and compact in habit; one (series 133) has white flowers and is very early, while the other (series 34) has deep-red flowers and is medium early. The other two races were obtained in California; both are pubescent, of open growth habit, and (except for the earlier form of series 39) late for annual stocks; series 38 is a purple-flowered form from mixed commercial seed, and series 39 is a pale-red race from a Riverside flower garden.

Miss SAUNDERS (1911, 1924) has identified two dominant genes, *C* and *R*, which are necessary for the development of sap color, and two additional dominant genes, *H* and *K*, which, *together with C and R*, are neces-

sary for the development of pubescence; there is very close linkage between *Rr* and *Hh*. Series 38 and series 39 (sap-colored pubescent) therefore are *CCRRHHKK*. Since Snowflake (white glabrous) gives a monohybrid F_2 ratio for sap color in these crosses, it must lack either *C* or *R*, but not both; since it gives a dihybrid F_2 ratio for pubescence (about 9 colored pubescent: 3 colored glabrous: 4 white glabrous) when crossed with series 34 (colored glabrous), and a monohybrid ratio (about 3 colored pubescent: 1 white glabrous) with series 38 and 39, it must contain *KK;* it must therefore be either *ccRRHHKK*, *CCrrhhKK* or *CCrrHHKK*. Series 34 (colored glabrous) must be either *CCRRHHkk* or *CCRRhhKK*.[3] Snowflake carries the dominant gene *B* for purple (Miss SAUNDERS 1911, 1924; TSCHERMAK 1912), and also a recessive gene for intense flower color (evidently *p;* Miss SAUNDERS 1911), which is linked with the genes for single and double flowers. Light purple and deep purple (series 38) are *BBPP* and *BBpp*, pale red (series 39) is *bbPP*, and deep red (series 34) seems to be *bbpp* plus further intensification.

Series 133 gave glabrous white single F_1 hybrids; the other three pure single races gave pubescent purple single F_1 hybrids. No clearly genetic variation not to be ascribed to extra chromosomes was observed within any F_1 hybrid family, or within any of the small families grown from selfing of the types used in crossing. With series 39, several types differing in earliness and leaf size were used in crossing, but these appeared to be homozygous for all characters observed. With series 38, a light purple (*BP*) and a deep purple (*Bp*), both homozygous, were crossed with Snowflake, but the deep purple is represented only by one F_1 plant in the data for chromosome shape.

The variety Snowflake differs strikingly from all of the other parental varieties in the shape of the paired chromosomes at the first metaphase in pollen mother cells. Figures la and lb, compared with figure 2a, show this difference clearly. An attempt was made to choose lateral metaphase figures in which the chromosomes were at their maximum elongation. If one were to compare such a figure from Snowflake with the most contracted figures seen in the pollen mother cells of any of the pure single varieties which entered into the crosses, the contrast would be far greater. In the examination of aceto-carmine smears for chromosome shape every plant can be classified with certainty, since while each type varies considerably, the majority of the pollen mother cells from the pure single varieties have exceedingly short chromosomes, and none has chromosomes

[3] The F_2 ratios (approximating 9:3:4) show that, if series 34 is *CCRRhhKK*, Snowflake must be *ccRRHHKK*.

as long as the longest in Snowflake. Snowflake pollen mother cells, on the other hand, characteristically have elongated chromosomes, and none of them has the short dumb-bell shape typical of the pure single races. These statements apply equally to the short-chromosome and long-chromosome types, respectively, among the hybrids. On account of the great length of the chromosomes in Snowflake pollen mother cells at first metaphase, it is impracticable to make counts from polar views in that variety, but this can often be done in the pure single varieties studied, since their chromosomes often approach the familiar dumb-bell shape. At later stages—first anaphase, second metaphase and second anaphase—the differences in shape are far less striking, although the chromosomes usually seem to be somewhat more compact in the pure single varieties than in Snowflake (figures 1c to 1e and 2b to 2d). Comparison of chromosome size at these stages and in somatic cells (root tips; figures 1f and 2e) shows that the actual amount of chromatin present is practically the same in the two parental types in spite of the very considerable differences in shape during the first metaphase. This is in accord with the fact that the pollen mother cells are of about the same size in all types examined.

That the short type of chromosome is dominant in the F_1 is shown by table 1, and by figure 3a, which, like figures 1a, 1b and 2a, was selected

LEGEND FOR FIGURES 1–4

The chromosomes were drawn at a magnification of 4000 diameters. Drawings reduced one sixth in reproduction.

FIGURE 1.—(a) Chromosomes of a pure single variety (series 34, plant 25.5–4), at first metaphase, showing contracted or short form. Of the 62 F_2 plants examined for chromosome shape, 33 were hybrids of series 34 with Snowflake.

(b) Same for another pure single variety (series 38, plant 25.6–1), except that chromosomes have been separated for clearness.

(c) First anaphase of plant 25.6–1.

(d) Polar view of one second-metaphase group of same.

(e) Lateral view of one second-anaphase figure of same.

(f) Somatic metaphase from root-tip cell of plant 25.5–4.

FIGURE 2.—(a) Lateral first metaphase of Snowflake variety. Chromosomes have been separated for clearness. Note great elongation as compared with figures 1a and 1b.

(b) First anaphase of same.

(c) Polar view of one second-metaphase group of same.

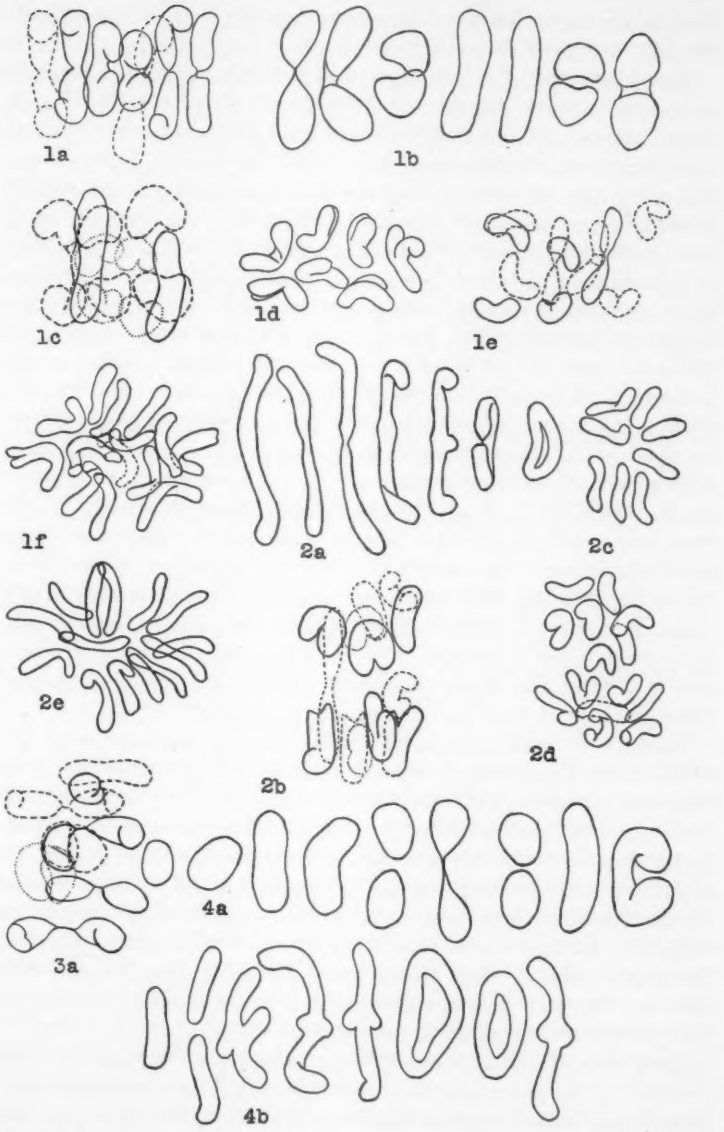
(d) Lateral view of second-anaphase group of same.

(e) Somatic metaphase from root tip of same.

FIGURE 3.—(a) Early first anaphase from an F_1 plant.

FIGURE 4.—(a) Lateral first metaphase of an F_2 plant with short chromosomes (25.21–30; Large Smooth, double trisomic type.)

(b) Same of an F_2 plant with long chromosomes (25.19–51; Narrow trisomic type). The unpaired extra chromosome is not comparable with the one at the extreme left in figure 4a, because the extra chromosome of Narrow is typically larger than that of Large.


1a
1b
1c
1d
1e
1f
2a
2c
2e
2b
2d
3a
4a
4b

to show the chromosomes at the greatest extension which was seen during the first metaphase in pollen mother cells.

Non-disjunction, fragmentation and non-reduction are always found in smears of pollen mother cells from Snowflake plants with 7 pairs of chromosomes. Of 675 pollen mother cells from one greenhouse plant examined after cytokinesis, 27 had formed dyads and 14 triads, and 15 had given rise to four microspores and one microcyte—abnormalities totaling 8.3 percent of the number of mother cells. On the other hand, the pure single varieties used in the crosses show extremely little abnormality of reduction or of tetrad formation. Rarely, in the short-chromosome races, one pair of chromosomes separates either before or after the rest at first metaphase and as a result one occasionally sees a chromosome which has been left out of a nucleus following the first division. A greenhouse plant of series 34, comparable with the Snowflake plant mentioned, showed, in the groups from 308 pollen mother cells, only 2 microcytes and no dyads or triads—a proportion of abnormality of only 0.6 percent. This great contrast in amount of abnormality appears to be typical. In the F_1 plants, in which also the chromosomes have the greatly contracted form characteristic of the pure single varieties, cytological abnormalities are similarly rare. This may mean that the contracted condition of the chromosomes in the pure single varieties and in the F_1 is associated with closer pairing, or "stronger affinity," than that which characterizes the long-chromosome Snowflake variety. The closeness of pairing and normality of reduction in the F_1 further indicate that the chromosomes of these varieties of Matthiola are strictly homologous.

Miss ALLEN (1924) compared double-throwing and pure single strains of *Matthiola incana* and found the cytology to be identical in all forms investigated. She found "no evidence of any irregularities in distribution such as is described so frequently among Oenothera hybrids." From her figures and the text it appears that both the eversporting (sulphur-white) and the pure single varieties studied had the short or dumbbell-shaped chromosomes which we have found to be characteristic of our pure single varieties. In this and in the regularity of its chromosome distribution the sulphur-white variety differs from Snowflake. It is therefore evident that the elongated type of chromosome is not necessarily associated with the "double-throwing" genic constitution.

Since such differences in chromosome shape may be found in different varieties of the same species of plants it would be impracticable to use chromosome length in pollen mother cells as an indication of chromosome size in a species, unless all varieties had been ascertained to be alike in

chromosome extension. It would obviously be better to use chromosome volume in comparative studies of this type.

Table 1 shows the numbers of plants examined for chromosome shape in the pure single races and in the hybrids. It should be added that many Snowflake plants have been examined cytologically, including normals and plants with one, two and three extra chromosomes, and no individual with short chromosomes has been found. With the pure single races, three generations are represented among the 8 plants examined; with series 38, two P_1 color types are represented by F_1 hybrid progeny examined; with series 39, one P_1 habit type is represented by F_1 plants examined, and two types in the other two cases. Aside from Snowflake and part of its F_2 hybrids, we have not found long chromosomes in any Matthiola plants.

TABLE 1

Inheritance of first-metaphase chromosome shape. Numbers of plants with short and with long chromosomes, in four pure single races (short) and in hybrids of these with Snowflake (long).

GENERATION	CHROMOSOMES SHORT					CHROMOSOMES LONG				
P_1 *series**:	133	34	38	39	All	133	34	38	39	All
P_1 type (pure single)	2	2	1	3	8	0	0	0	0	0
Hybrid F_1	3	7	4	4	18	0	0	0	0	0
Hybrid F_2	..	23	1	21	45	..	10	3	4	17

* These are the pure single races employed in crossing. In all the crosses, one P_1 race was Snowflake (long chromosomes), trisomic forms of this variety being used as parents.

THE F_2 HYBRIDS

The crosses involving series 34 give the expected segregation and recombination of color and pubescence characters in F_2, producing purple and red pubescent and purple and red glabrous, both with several grades of color intensity, and white glabrous; the compact habit of series 34 also reappears, apparently as a simple recessive character. The crosses of series 38 (light-purple form) give purple pubescent and white glabrous, and those of series 39 give purple pubescent, red pubescent, and white glabrous; in both cases the recessive intense form *pp* (deep purple or light red, as contrasted with the light purple or pale red produced when *P* is present) appears. F_1 hybrids from a late form of series 39 produce in F_2 a late type with large leaves, which is very distinct under some conditions and may be a simple recessive.

Clearly the hybrids of Snowflake with these pure single races show in F_2 the typical Mendelian segregation for color and pubescence which

Miss SAUNDERS (1924) and TSCHERMAK (1912) have so extensively demonstrated for this species. Part of the F_2 families, as expected, also include the recessive double-flowered type; since, however, the doubles have no stamens, only singles could be used in the determination of chromosome shape.

Only about one-fourth of the 62 F_2 plants classified for chromosome shape in table 1 were chosen, in connection with the present study, to represent various somatic segregation types; the rest were chosen primarily either for special study of trisomy and tetrasomy among the hybrids, or simply for determination of chromosome number to check the classification of mutant types. On account of the method of selection of plants for the determination of chromosome shape, the ratios for the various other segregating characters have no significance.

As tables 1 to 3 show, all plants examined for chromosome shape in the F_2 had either long (elongated) chromosomes like Snowflake or short (contracted) ones like the pure single varieties used in the crosses. Here, as in the parental varieties, there has been no case of questionable classification. The long-chromosome character appears to be a simple recessive, appearing in only 17 out of the 62 plants examined. The data for chromosome shape were accumulated during a period of several months, and they were never far from a 3:1 ratio after 12 plants had been examined.

Figures 4a and 4b show chromosome-shape differences between an F_2 plant with short and one with long chromosomes at first metaphase. Figure 4a shows the very compact condition which is usual in short-chromosome plants but which is never approached in plants with long chromosomes. The chromosomes in figure 4b are not exceptionally long for F_2 long-chromosome plants.

In the F_2 as in the parent varieties and the F_1, reduction was relatively normal in pollen mother cells from plants with short chromosomes even when odd chromosomes were present in addition to the normal seven pairs, whereas abnormalities of reduction similar to those found in Snowflake were always found in the pollen mother cells of the segregants with long chromosomes.[4] It seems probable that the elongation of the chromosomes is dependent upon a single gene and that normality of reduction is either directly or indirectly controlled by it also. Possibly the elongated form entails mechanical difficulties in the conjunction and disjunction of the chromosomes. It may also favor fragmentation.

[4] Since the F_2 data on abnormal cytokinesis were mainly obtained from trisomics, detailed presentation is reserved for a general report on the cytology of these forms.

Snowflake plants with the normal complement of chromosomes are remarkable for their abundant production of trisomic progeny (FROST 1919). Although we have no adequate records from short-chromosome races or their F_1 hybrids for comparison, it seems highly probable that these will produce much smaller percentages of chromosomal mutants. In view of the extensive and thorough study of Matthiola by Miss SAUNDERS (1924 and papers there cited) and by TSCHERMAK (1912), it seems improbable that these workers would have failed to record variant forms so striking and so abundant as these mutants have proved with Snowflake. DE VRIES (1906, p. 338), however, describes one form suggestive of chromosomal mutation; the "stiff and erect appearance" of these variants (called "generals" in Erfurt) suggests our Large trisomic, although the other characters mentioned, especially the "more luxurious growth" hardly favor this identification.

Since the gene for long appears to be exceptional in the species, it seems likely that it arose from its "short" allelomorph by mutation. It is therefore appropriate to use the symbol *l* for long and *L* for short.

The two chromosome types in F_2 were well distributed among the various trisomic and segregant types (tables 2 and 3). The recombinations of characters seem to demonstrate that gametic recombination for chromosome shape has repeatedly occurred.[5] The F_1 hybrids derived from series 34 and 39 (red corolla) received the gene for purple from the Snowflake parent only. Therefore, the genes *B* (purple) and *l* (long) entered these hybrids from the same parent, and the one red long (*bbll*) F_2 plant of table 3 indicates separation of *B* and *l* in gametogenesis. In a similar way, the ten white shorts (*rr* or *cc* plus *Ll* or *LL*) indicate that *L* separated from a gene for color (presumably Miss Saunders's *R* or *C*). All the colored longs (*BBll*, *Bbll* or *bbll*, with *R* and *C*) also indicate separation of *L* from *R* or *C*. Two of the three intense shorts (*ppLl* or *ppLL*) from series 39 indicate separation of *P* and *L;* the third is doubtful, since this plant was an extreme (tetrasomic) Slender and doubtless had *two* extra chromosomes (fragments) derived from the intensity-carrying (*PP*) pair of Snowflake. It is therefore evident that the *L* and *l* genes recombine with *Bb*, *Pp* and *Rr* or *Cc*.

[5] Complications due to trisomy may invalidate this conclusion in an occasional case. For example, a plant of the constitution $\frac{lCR}{LCR}$ would be colored short, but the addition of an extra chromosome carrying *l* but not the color genes would give colored long, providing *ll* is dominant over *L*.

Doubleness, a recessive character for which all Snowflake singles are heterozygous, is not represented in the data for chromosome shape, because doubles lack stamens. In F_2 progenies segregating for doubleness,

TABLE 2

Numbers of F_2 plants, with short and with long first-metaphase chromosomes, belonging to various trisomic and tetrasomic types.

	CRENATE	DARK	LARGE	NARROW	SLENDER	SMALL	SMOOTH	LARGE SMOOTH	NORMAL	ALL TYPES
Chromosomes short	4	2	2*	1	5†	8†	6†	1	16	45
Chromosomes long	3	0	0	1	3	4†	3	0	3	17

* Both tetrasomics. † Including one tetrasomic in each case.

however, all single longs indicate separation of *l* from a gene for double. These progenies gave the ratio 26 short: 8 long, while the all-single progenies, derived from F_1 plants homozygous for single, gave the ratio 19:8. *L* and *l* therefore recombine freely with the single-double allelomorphs.

TABLE 3

*Relation of first-metaphase chromosome shape to other segregating characters in F_2. Numbers of plants with the characters indicated.***

SOMATIC CHARACTERS	CHROMOSOMES SHORT				CHROMOSOMES LONG			
P_1 series:	34	38	39*	All	34	38*	39	All
Pubescent purple	8	1	10 (2)†	19	7	3 (1)	3	13
Pubescent red	3		8 (1)	11	1			1
Glabrous purple	3			3				
Glabrous red	1			1				
Glabrous white	7		3	10	2		1	3
Totals	23‡	1	21 (3)	45‡	10	3 (1)	4	17

* The F_2 hybrids from series 38 and 39 which showed the recessive intense color (*pp*) derived from Snowflake are indicated by the numbers in parenthesis.

† One of these intense shorts was extreme (presumably tetrasomic) Slender.

‡ Including one feeble tetrasomic, on which no open flowers were observed.

** The following data, for normal F_2 plants, progeny of normal F_1 plants heterozygous for red, have been obtained too late for inclusion in table 3: purple, 11 short:4 long (homozygous purple, 7:3); red, 7:5. In addition, 5 miscellaneous F_2 plants were all short. The F_2 totals are therefore now: 68 short:26 long.

In the case of the F_2 plants descended from the later form of series 39, no recombination for lateness was demonstrated; the 5 early (dominant) and 7 late plants examined all had short chromosomes. This absence of the double recessive form following a "repulsion" cross (late short×early long) suggests linkage, but several times as many late plants would be

necessary to give this absence much statistical significance. Of the 14 F_2 descendants of series 34 whose chromosomes were examined last (late in 1926), 13 had been classified for the recessive compactness derived from that parental race; 4 of these were compact, but none were long, and therefore no recombination was demonstrated.

Table 3, with the late data added in the last footnote, includes 46 purples and 25 reds derived from F_1 parents heterozygous for red. There is no significant indication of linkage between *Bb* and *Ll*, for the ratios are: purple, 32 short:14 long; red, 19:6. The purples (ratio 7:3) listed as homozygous were determined by examination of the seed (embryo) color in their capsules.

SUMMARY

1. One horticultural variety of *Matthiola incana* R. Br has chromosomes which are remarkably long at the late prophase and the metaphase of the first meiotic division in the pollen mother cells. Other races have much shorter first-metaphase chromosomes.

2. The long-chromosome condition is recessive in F_1 hybrids and has reappeared in F_2 in about one-fourth of the individuals. It therefore seems to be due to a single gene, which may be denoted by *l*.

3. Normal plants of the long-chromosome variety produce several percent of trisomic progeny, of at least eight kinds. In three of these mutant types the extra chromosome is clearly a fragment of a normal chromosome.

4. Abnormal chromosome behavior has frequently been observed in the pollen mother cells of the long-chromosome race. In the short-chromosome races, on the other hand, reduction and tetrad formation are almost always normal. This is also true of the F_1 hybrids between long and short, and of the F_2 plants with short chromosomes, but the F_2 segregants with long chromosomes show much the same amount and type of abnormality of reduction as the long-chromosome variety. Evidently the long-chromosome gene in some way favors abnormalities of meiosis—possibly as a direct result of the length and attenuation of the chromosomes.

5. Three genes for color characters, which other workers have found to exhibit typical Mendelian behavior in hybrids, have given evidence of recombination with the genes for chromosome shape. The genes for chromosome shape appear not to be linked with those for single *versus* double flowers.

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